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LI. ENZYME-ACTION, FACTS AND THEORY.

By HENDRIK PIETER BARENDRECHT.

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(*Received August 30th, 1913.*)

The researches of the last few years on the kinetics of enzyme action have brought more confusion than clearness into this field. Even about the action of the best studied enzyme, invertase, there is contradiction and uncertainty; it seems as yet not quite established if, or under what conditions, the hydrolysis of cane-sugar by invertase follows the simple law of mass-action.

As to the nature of enzyme action there is a general inclination to suppose, that some chemical combination of enzyme and substrate plays a leading part in the process and that the general properties of the colloids, to which enzymes seem to belong, will supply sufficient explanation of their remarkable activities.

The aim of this paper is to clear up some of the contradictory statements in the literature about the kinetics of the most simple enzyme actions, and to show at the same time that there is another theory, which is more in accordance with the peculiarly characteristic behaviour of enzymes.

The hydrolysis of cane-sugar by invertase has been chosen by many investigators as the best process on which to study the velocity of enzyme action in relation with the different factors, which are here of importance. Both enzyme and hydrolyte are readily accessible, the action is simple, starts with the well-known substance saccharose and leads to the also fairly well-known substances glucose and levulose.

Chemically speaking, this hydrolysis is a monomolecular reaction. It was therefore a confirmation of what chemists expected, when O'Sullivan and Tompson [1890] found in an elaborate investigation, that the inversion of saccharose by invertase from yeast proceeded in accordance with the law of mass-action, i.e. if y represents the inverted fraction at a time t, $k = \frac{1}{t} \log \frac{1}{1-y}$ was nearly a constant.

Duclaux [1898] however, without doubting the correctness of the experimental results of O'Sullivan and Tompson, pointed out, that there was still a remarkable difference between the action of acids and invertase (and of enzymes generally).

In differently concentrated solutions of saccharose the same amount of acid gives the same velocity-constant k, thus for instance a 5, 10 or 15 % saccharose solution will be inverted by a 1 % acid solution in the same time to the same extent, e.g. half way. But for invertase Duclaux had found as early as 1883, that the same quantity of enzyme, acting in different concentrations of cane-sugar, inverted in the same time not the same fractions but the same absolute amounts.

Henri [1901] then took up again the experimental study of invertase and found, that not only was there the difference between the actions of enzymes and acids, established by Duclaux, but that k did not even remain constant during one and the same action of invertase on cane-sugar; on the contrary steadily increased.

Now it cannot be denied, that Henri's experiments were open to criticism owing to his not taking sufficiently into account the mutarotation of the freshly formed glucose in his polarimetric estimations.

It was therefore a valuable contribution on the experimental side of the subject, when A. Brown [1902] also investigated the kinetics of the invertase-action and found the same regular increase of the constant $k = \frac{1}{t} \log \frac{1}{1-y}$ for sufficiently concentrated saccharose solutions as Henri. On p. 375 of his article Brown says: "Conditions of experiment similar to those used by C. O'Sullivan and Tompson were employed, but the invertase used was prepared in a different manner from the enzyme with which these authors experimented."

To this last point we will return further on.

In order to remove all doubt, possibly also expressed by others as to the correctness of Brown's estimations, I wrote to him on this subject and was assured, that he "was well aware of O'Sullivan's experiences with regard to mutarotation and took exactly the same precautions to avoid errors in connection with it, which O'Sullivan adopted."

As I myself [1904] found the same deviation from the simple law of mass-action as Brown, without using a polariscope at all, but estimating the hydrolysis of cane-sugar by the accurate gravimetric method of Kjeldahl with Fehling's solution, Hudson's [1908] criticism of his predecessors (excepting Henri) must be rejected. How it was, that Hudson could again find validity

of the simple formula of mass-action in his experiments with invertase was explained by the work of Sörensen [1909].

In his elaborate article on: "The measurement and importance of the concentration of hydrion in enzyme-action" this author shows, that low concentrations of hydrion in the invertase-solution give the well-known increasing k. Going beyond this concentration one can work with an invertase-solution, where the reaction velocity follows the formula

$$k = \frac{1}{t} \log \frac{1}{1-y},$$

and in still more distinctly acid solutions k diminishes even more and more rapidly during the reaction. That this irregularity in the reaction-velocity is due to a gradually decaying of the enzyme by the action of the hydrion was perhaps not sufficiently emphasised in this article, though it is clearly expressed on p. 146 for the series with the highest hydrion concentration, where the reaction did not even come to a finish.

Indeed it follows from Sörensen's observations and deductions, that it is dangerous to work with enzymes at their optimum concentration of hydrion as well as at the optimum temperature of their activity. Both factors in that condition give splendidly rapid enzyme action, but at the same time are gradually destroying the enzyme itself.

It is obvious, that, where the quantity of the active catalyst is diminishing during a reaction, the velocity of this reaction will diminish at the same rate and may thus seem to fulfil the simple formula of mass-action. With low hydrion concentration, that is without adding acid or using extracts that have become acid by deterioration, one always gets for invertase (provided the saccharose solution is sufficiently concentrated) the same increase in the constant k, calculated as $k = \frac{1}{t} \log \frac{1}{1-y}$, or a real constant k, when it is calculated according to the empirical formula of Henri or to the theoretical formula, which I developed in 1904.

Sörensen drew attention to the fact, that O'Sullivan and Tompson brought their solutions by the addition of sulphuric acid to about the maximum of activity of the invertase, while Hudson worked with solutions containing acetic acid. Now we can add to this, that Brown "employed in (his) experiments an extract of invertase, prepared from dried yeast by digestion with water," and "without the complicating addition of sulphuric acid." In my own experiments I used an extract of fresh yeast, mixed with kieselguhr and dried rapidly at a low temperature. No acid at all was used.

When examining the results of O'Sullivan and Tompson as closely as these authors themselves did, one sees clearly, that the acidity they employed had not reduced the real velocities quite to an extent sufficient for the law of simple mass-action to be perfectly imitated. On p. 846 they say: "But if we look more closely at the diagram, we see that the deviations from the theoretical line, though small in themselves, are constant in the three experiments; in fact they correspond in a very striking manner." And a little further: "These figures all show a continuous increase in activity up to a certain point, about 80 per cent. of inversion, and after that a large and increasing decrease." That is exactly what the gradual destruction of the invertase must produce.

Sörensen goes however too far, when he generalises this influence of hydrion concentration to suggest a sufficient explanation for the marked falling off of the constant $k = \frac{1}{t} \log \frac{1}{1-y}$ in the experiments of Armstrong with lactase. My own experiments as well as the theory of enzyme action, which I proposed some years ago, lead to a different explanation and to a real reaction-constant in this case also.

As the application of this theory may now be extended in some points beyond what was published before, it may be of interest to survey once more a part of this much entangled field of enzyme actions with the aid of this theory.

Any hypothesis of enzyme action must be founded on the principal characteristics of this kind of catalysis.

It seems more and more established that these are:

1. That the same quantities of enzyme, brought into differently, but sufficiently, concentrated solutions of substrate, produce in the beginning the same action in the same time.

2. That catalysis by enzymes has a marked specific character[1].

Starting from these general facts, I ventured the hypothesis [1904] that enzyme action spreads like radiation from an enzyme particle as centre, and that an enzyme particle contains the same molecule, which is liberated or acted upon by this enzyme, in some active state.

What kind of radiation this may be cannot yet be decided. It is however very remarkable, that an imitation of specific enzyme actions has been produced by Rosenthaler [1908] by electromagnetic oscillations. By placing

[1] This specificity is known to enzyme workers to be probably not so absolute as is often taken for granted. According to the following theory a limited specificity must also be looked for.

his substrate solutions inside a solenoid, through which a frequently interrupted direct current or an alternating current was passed, a diastase-like action on starch was observed for a frequency of 440–480 per second, and a hydrolysis of proteins for one of 320–360.

In order to explain the principal facts it is necessary to make the natural assumption, that this radiation may be absorbed by the substrate itself, by the substances liberated from it by the enzyme action, or by any other foreign substance added to the solution. The possible absorption by the water itself must be practically the same in every case and can therefore be left out of account.

It is evident that the sphere, within which this radiation-like effect around an enzyme particle may be expected to be active, must be very small; otherwise enzyme actions would be instantaneous.

In the beginning the spheres of action in differently, but sufficiently concentrated solutions of substrate will be inversely proportional to that concentration; therefore in the first time-unit the same quantity of enzyme will produce the same amount of effect in these solutions, for practically all radiation is absorbed by the substrate only.

In more dilute solutions not all the radiation will reach a substrate particle before it is too much weakened by spreading. This explains the fact that in moderately dilute solutions the constancy of the amount of initial action has disappeared. In sufficiently dilute solutions of substrate we shall practically obtain the condition, that on the average no two particles of substrate are on the same radius of the radiation sphere, so that all these particles will be acted upon at the same time and the amount of action will be directly proportional to the concentration of substrate; a fact observed by myself [1904] as well as by many other investigators.

The advantage of a reasonable working hypothesis proves here again to be its giving an indication, where to look for regularities and where these are not to be expected.

This hypothesis can also be quantitatively developed and leads, as I have shown, to a theoretical formula for the reaction-velocity in sufficiently concentrated solutions of substrate. We shall call the initial concentrations of the substrate a, the same after a time t of reaction x (all expressed for instance in grammes per 100 c.c.), and the amount of action in the first time-unit by a given quantity of enzyme in 100 c.c. of these sufficiently concentrated solutions m. According to theory as well as to experiment m is independent of a.

If further, n is the ratio between the absorption power for the enzyme

radiation of the reaction-products and that of the original substrate, the general differential equation for the rate of reaction is:

$$-dx = m\frac{x}{x+n(a-x)}dt.$$

As however catalysis by enzymes is generally recognised to be as a matter of fact a balanced action, this formula for the rate of reaction can only be applicable, where there is evidence that the reverse action, the synthesis, does not come into play. It will be shown further on, that this happens to be the case as a rule in the experiments with two of the best studied and relatively simple enzymes, invertase and lactase.

Bearing in mind this restriction, we obtain by substitution of $\frac{a-x}{a} = y$ and integration, the general formula for the rate of enzyme action in sufficiently concentrated solutions of substrate:

$$\log\frac{1}{1-y} + \frac{1-n}{n}0{\cdot}434y = \frac{m}{na}0{\cdot}434t$$

expressed in decimal logarithms.

The two constants in these experiments can be determined in different ways.

They may be calculated from the observed action at two different time-intervals, or m can be directly found by estimating the change during the first time-unit and n from the same estimation in similar conditions, but in a solution, in which had also been dissolved a known amount b per 100 c.c. of the reaction-products.

In the first estimation the initial velocity will be

$$\left(\frac{dy}{dt}\right)_{y=0} = \frac{m}{a},$$

in the second, from

$$-\frac{dx}{dt} = m\frac{x}{x+n(a-x)+nb}dt,$$

we get

$$\left(\frac{dy}{dt}\right)_{y=0} = \frac{m}{a}\frac{1}{1+n\frac{b}{a}}.$$

Evidently the value of n will depend only on the nature of enzyme and substrate, and must therefore be the same in the experiments of different observers.

For invertase from ordinary yeast the estimations of all the investigators, who avoided the errors mentioned in the first part of this article, agree with the value $n = \frac{1}{2}$, and I found the same also by direct estimation of initial velocities.

As $(a-x)$ grammes saccharose give by inversion $\frac{360}{342}$ grammes invert-sugar, the rate of inversion of cane-sugar in sufficiently concentrated solutions by unaltered invertase is thus generally represented by the equation:

$$\log \frac{1}{1-y} + 0{\cdot}393y = kt.$$

The enzyme lactase is not as readily accessible as invertase, therefore its action is not so often studied. Ordinary brewers- or distillers-yeast is inactive in regard to milk-sugar. It is necessary to employ the special yeast, which is known to be the principal active agent of "kephir."

In order to extract the enzyme lactase as fresh and as unaltered as possible from the living cell, which produces it, I prepared a sufficient quantity of a pure culture of "Saccharomyces kephir" and worked only with the fresh extract from this yeast, which had been mixed with kieselguhr and dried in vacuum at a low temperature [1906].

In estimating the velocity of inversion of an 8 % milk-sugar solution at 30° by this lactase, I found a series of figures, to which it will now be shown that the general equation:

$$\log \frac{1}{1-y} + \frac{1-n}{n} 0{\cdot}434y = \frac{m}{na} 0{\cdot}434t$$

can also be applied with the same success.

Only, as explained above, the value of n may be expected to be different from that for invertase.

Direct observations of the very large retarding influence of glucose and of galactose showed that n is here much greater than in the case of invertase.

Calculation from two estimations of the hydrolysis of lactose at different time-intervals gives about $n = 6$. Thus for lactose the velocity of inversion is represented by

$$\log \frac{1}{1-y} - 0{\cdot}362y = kt.$$

For 8 % milk-sugar at 30° I found, estimating the hydrolysis again by the gravimetric Fehling-Kjeldahl method:

t (minutes)	y	$k = \frac{1}{t}\left(\log \frac{1}{1-y} - 0{\cdot}362y\right)$
5	0·23	0·0061
10	0·38	0·0070
15	0·47	0·0070
20	0·57	0·0080
25	0·61	0·0075
40	0·72	0·0073
66	0·82	0·0068
90	0·90	0·0075

Armstrong [1904], though also emphasising the importance of working rapidly and using only fresh extracts, did not it seems give care enough to these precautions. Kephir-grains, an extract of which he used, are a mixture of kephir-yeast cells with lactic acid bacteria and other residues, all together a mixture of somewhat unknown history and origin. The extract will contain some acid and in fact was not very active, compared with that obtainable from fresh pure lactose yeast. The experiments lasted several hours and even days, so that it was necessary to add toluene. The risk of decaying of the enzyme during these long experiments at 35°–37° was therefore not sufficiently excluded.

Still the first series of his investigations (p. 506) calculated with our lactose equation, gives

t (hours)	y	$k=\frac{1}{t}\log\frac{1}{1-y}$	$k=\frac{1}{t}\left(\log\frac{1}{1-y}-0{\cdot}362y\right)$
1	0·221	0·1085	0·0285
2	0·312	0·0812	0·0247
3	0·384	0·0713	0·0244
4	0·458	0·0665	0·0250
5	0·515	0·0629	0·0256
6	0·566	0·0604	0·0263
10	0·69	0·0509	0·0259
17	0·842	0·0471	0·0290
23	0·924	0·0461	0·033
29	0·953	0·0457	0·034
38	0·98	0·0447	0·035

The last of these estimations are less trustworthy, owing to exposure of enzyme and of milk-sugar to the combined action of the slight acidity and the temperature of 35°.

The other series of Armstrong's article lasted still longer, the inversions were far from finished at the end, and proceeded only very slowly during the last 24 hours. Evidently the enzyme was here to a large extent destroyed.

There are further regularities in the working of these relatively simple enzymes, invertase and lactase, which only become apparent when the investigator takes sufficient care to work only with enzymes, extracted with caution directly from the fresh living cells.

As I have shown before, the experiments not only agree with the conclusion, drawn from our hypothesis, that generally all neutral substances as well as the inversion products must retard the enzyme action, but striking quantitative regularities also exist.

For *unaltered*[1] invertase from ordinary yeast the facts are these:

[1] By drying the yeast at a high temperature or treatment with alcohol I got an invertase, the action of which was more retarded by levulose than by glucose.

If we represent the retarding power (or the absorption coefficient according to our conception) of glucose by $n = \frac{1}{2}$, then the retarding powers of levulose and of invert-sugar are found to be exactly $n = \frac{1}{2}$ also. For the retarding powers of galactose and mannose however the experiment gives the double value $n = 1$. Nearly all neutral substances give retardation of the same order as the sugars, e.g. dulcitol, mannitol, urea, salicin and other glucosides.

It is very remarkable, that milk-sugar alone does not appreciably retard the action of invertase. In connection with this may be mentioned the small absorption power that milk-sugar was also shown to have for lactase action, compared with that of all other sugars. The figure for the inversion couple from milk-sugar was found to be $n = 6$. Galactose retards twice as much as glucose, so for galactose the absorption power of lactose action is $n = 8$, that of glucose $n = 4$. For levulose, a hexose which is here not contained in the substrate, experiment gives also the sum of the absorption powers of the inversion products of milk-sugar, that is $n = 12$.

In connection with the recent work of Tanret [1905] and of Hudson [1908] I wish to draw attention here to another point about the action of invertase and maltase.

If enzymes, as seems now more and more probable, are able to decompose a substance as well as to actuate the reverse process, then invertase, maltase and lactase should produce an equilibrium between bihexose and hexoses. In the case of maltase Croft Hill [1898] seemed to have established some synthesis of maltose from glucose, but it was proved afterwards, that the bihexose formed with difficulty was, at least for the most part, another substance. As for invertase and lactase, the evidence of synthesis of cane-sugar and milk-sugar is even slighter.

The facts, that a fairly concentrated solution of cane-sugar is practically completely hydrolysable by means of invertase and that after all no appreciable enzymatic synthesis of maltose from glucose is observed, suggest the conclusion, that the hexoses, split off by these enzymes, undergo a secondary change, by which the reverse enzyme action is prohibited.

Through the investigations of the mutarotation of sugars, especially by Tanret and by Hudson, much clearness has been brought into our knowledge of the isomerism of the most important members of this group. It was proved by Tanret, that glucose, dissolved in water is at first totally in the α-form ($[\alpha]_D = 110°$) and then gradually changes into a mixture in equilibrium ($[\alpha]_D = 52{\cdot}5°$) of about two-thirds α-glucose and one-third β-glucose ($[\alpha]_D = 19°$).

As two-thirds of glucose in solution are finally in this α-modification, then, if the glucose, split off by enzyme action from cane-sugar or maltose should be originally in this same form, as is generally assumed, it is difficult to see why the reverse action of the enzyme should not produce a considerable amount of maltose.

Also the hydrolysis of cane-sugar by invertase should then not be expected to come to a finish.

The rate of mutarotation of this α-glucose, i.e. the velocity with which it comes to this equilibrium, was carefully measured by Hudson, who made a similar investigation of the mutarotation of levulose. Combining these investigations with his experimentally quite correct estimation of the velocity of inversion of cane-sugar by invertase, Hudson came to the conclusion, that it is really α-glucose, which is originally formed by enzyme action in this case.

As the mean value of 18 experiments he found however for the specific rotation of this "fresh" glucose 120·7°, while this figure for α-glucose is 110°. This difference is so considerable, that Hudson's work also seems to point to the non-identity of enzyme-made fresh glucose with α-glucose.

This result is in complete agreement with the experimental evidence I got in 1904 of an unstable form of glucose, which seems to be the primary product of enzyme action and which gradually is converted to ordinary glucose in solution, that is, as we know now, to a mixture of α-glucose and β-glucose, according to the new nomenclature of Tanret.

It was expected, if the inversion of maltose was accomplished by a very active maltase solution[1] rapidly enough for the velocity of inversion to be great, compared with the velocity of conversion of the freshly formed glucose, that after the real equilibrium point maltose$\rightleftarrows$glucose had been reached, the rate of inversion would appear to be approximately constant.

The curve, which must result from these two simultaneous reactions, may be demonstrated with the aid of Fig. 1.

After the few minutes that are needed for the principal reaction to come to the real equilibrium point P, the relatively slow secondary change of unstable to stable glucose has practically not yet started. Being a monomolecular reaction, this rate of change of glucose will be at any time proportional to the concentration of unchanged unstable glucose.

As soon however as the concentration of unstable glucose has been

[1] In my article of 1904 I omitted to mention, that these very active maltase extracts of the dried yeast-kieselguhr were obtained by using water with a small amount of sodium hydroxide. Extraction with water only gave a maltase solution of smaller activity.

diminished by this change, the equilibrium between maltose and glucose is disturbed and part of the maltose is split again. As we have arranged the conditions so that this inversion of maltose is much quicker than the transformation of glucose, the concentration of unstable glucose and therefore the rate of its change will be maintained constant (in first approximation).

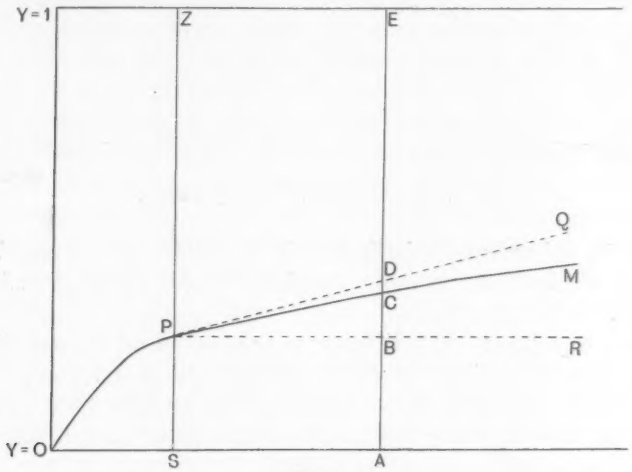

Fig. 1.

Thus in Fig. 1 we can represent, also in first approximation, the formation of stable glucose by the dotted straight line *PQ*, and the amount of unstable glucose by the horizontal dotted line *PR*. At any time, e.g. after *A* minutes, the equilibrium between maltose and unstable glucose will be immediately restored by the quick enzyme action. As however the concentration of maltose at the time *A* is somewhat smaller than that at the time *S*, the concentration of unstable glucose, required to balance by synthesis the hydrolysis of maltose, is also somewhat smaller than *SP* or *AB*.

The unstable glucose which has disappeared therefore need not be totally replaced by newly inverted maltose and the result will be, that at the time *A* this inversion will not have proceeded exactly to *D*, but to *C*. The estimations of the inversion of maltose will thus give a curve *PM*, slightly bending downwards from the straight line *PQ*.

Fig. 2, which illustrates the type of result obtained in my experiments of 1904, may serve also here to demonstrate the concordance with the theoretical type of Fig. 1.

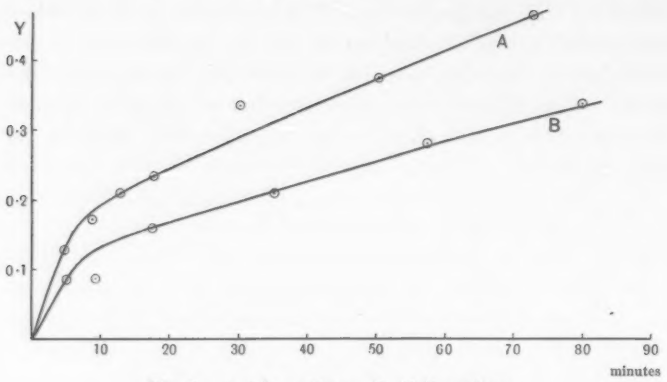

Fig. 2. A, 5 % maltose. B, 10 % maltose.

All the estimations of the hydrolysis of maltose were executed with Fehling's solution by the gravimetric method of Kjeldahl [1895] for mixtures of sugars.

These experiments with different concentrations of maltose showed further, that the positions of the equilibrium points, i.e. the points of flexure, where the curves become approximately straight lines, were connected together as these figures are in a balanced action between a monomolecular and a bimolecular reaction.

Following up the conception, that an enzyme particle extends its catalytic action in a sphere as regards both the reverse action and the hydrolysis of maltose, and that the size of this sphere in sufficiently concentrated solutions must be inversely proportional to the sum of the concentrations of maltose and glucose (the concentration of glucose multiplied by the absorption coefficient n), the complete formula for the velocity of action of maltase on maltose in conditions, where the secondary change of glucose is not yet perceptible, is

$$-dx = m \left\{\frac{x - q(a-x)^2}{x + n(a-x)}\right\} dt.$$

After substituting again $\frac{a-x}{a} = y$, the equation, relating the equilibrium points (where $-\frac{dx}{dt} = 0$) with the initial maltose concentration, is:

$$1 - y - qay^2 = 0 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (E).$$

For a solution of 10 grammes maltose in 100 c.c. for instance, the experiment gave about $y = 0{\cdot}15$, from which followed $q = 4$.

The other experiments with concentrations of 7, 5 (see Fig. 2), 3 and 1 g. for 100 c.c. agreed then with the figures, calculated from equation (E).

a	y
10	0·15
7	0·17
5	0·20
3	0·25
1	0·39

The synthesis of bihexoses in the living cell and the indications of synthesis in vitro, however slight they may be, suggest the conclusion, that the transformation of enzyme-made glucose to stable glucose is in fact also a balanced action, in which the equilibrium is far on the side of stable glucose.

In enzyme actions like this one, where the real equilibrium is so soon attained and the reverse action is thus vigorously hindering the hydrolysis, it is clear, that addition beforehand of the product of the reaction may retard more than that of any other compound of the same kind. The conversion of the unstable enzyme-made glucose is retarded considerably by the added glucose and with that the reverse action can assert itself.

For maltose I found a much larger retardation by glucose than by galactose and levulose.

Hence there is in fact no discrepancy between the peculiar retardation effects of foreign substances on the action of invertase and lactase on one side and the general rule, applied with success by ter Meulen [1905], on the other, that the enzymatic hydrolysis of a glucoside is most retarded by the same sugar, which is split off from the glucoside. The diagrams, representing the rates of hydrolysis of many of these glucosides, have the same character as that of maltose-inversion. Here also the reverse action would soon stop the further splitting of the glucoside, if the sugar, which is formed, were not liable to a secondary change, which change is effectively retarded by addition of a large amount of the stable form of the same sugar.

REFERENCES.

Armstrong (1904), *Proc. Roy. Soc.* **73**, 500.
Barendrecht (1904), *Zeitsch. physikal. Chem.* **49**, 456.
—— (1906), *Zeitsch. physikal. Chem.* **54**, 367.
Brown (1902), *J. Chem. Soc.* **81**, 373.
Croft Hill (1898), *J. Chem. Soc.* **73**, 634.
Duclaux (1898), *Ann. Inst. Past.* **12**, 96.
Henri (1901), *Compt. rend.* **133**, 891.
Hudson (1908), *J. Amer. Chem. Soc.* **30**, 1564.
Kjeldahl (1895), *Compt. rend. Carlsberg*, **4**, 329.
O'Sullivan and Tompson (1890), *J. Chem. Soc.* **57**, 865.
Rosenthaler (1908), *Sitzungsber. K. Preuss. Akad. Wiss.* 20.
Sörensen (1909), *Compt. rend. Carlsberg*, **8**, 120.
Tanret (1905), *Bull. Soc. Chim.* (3), **33**, 337.
ter Meulen (1905), *Rec. Trav. Chim.* **24**, 444.

LII. AN INVESTIGATION INTO THE PHYSICO-CHEMICAL MECHANISM OF HAEMOLYSIS BY SPECIFIC HAEMOLYSINS. (Preliminary Communication.)

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The mechanism of haemolysis by specific haemolysins has not been much investigated from the physico-chemical standpoint. It is supposed that the haemolysin affects the permeability of the envelope. Baumgarten has noticed that the first stage of haemolysis, as produced by a biological poison, is the swelling of the corpuscles, just as occurs when the serum is made hypotonic. Bang [1910] considers that haemolysis by means of Cobra poison is due to change in the composition of the lipoid membrane of the erythrocyte rendering it more permeable to salts. Through this membrane the extracellular salts and water soak gradually, until in the end the corpuscle ruptures. The behaviour of the nucleated erythrocytes of amphibians towards specific haemolysins tends to shew that their permeability is alone affected during the process [Landau 1903].

So far as I am aware, no work has been done on the behaviour of erythrocytes loaded with amboceptor towards distilled water before the complement has been allowed to act upon them. The present paper gives the results of investigations on the determination of the resisting power of amboceptor-loaded human erythrocytes to haemolysis when under the influence of distilled water. The haemolytic antisera were obtained from fowls and rabbits that had been immunised by means of injections of washed human corpuscles.

It was originally expected that amboceptor-loaded corpuscles would be less resistant than normal ones in their behaviour towards distilled water. Contrary to what was expected, I noticed a remarkable increase of the resisting power of the erythrocytes.

The method adopted for determining the resisting power of the erythrocytes is that previously described by me [1909, 1911] for the determination

of the specific resistance of erythrocytes to haemolysis. A series of experiments was performed and these are described as follows:—

In the first series, the erythrocytes were suspended in 0·85% NaCl and allowed to haemolyse with two parts of distilled water. The mixture was allowed to stand for ten minutes and the dissolved haemoglobin was estimated in the manner previously described. This gave the resistance of the normal erythrocytes to haemolysis.

In the second series, the erythrocytes were mixed with two parts of a 10% dilution of complement-free antihuman fowl's haemolytic serum with 0·85% NaCl and kept in the incubator for one to three hours and subsequently treated in the same way as above with distilled water after having been thoroughly washed in 0·85% NaCl, and the resistance of the amboceptor-loaded erythrocytes determined in the same way as above.

In the third series, the erythrocytes were treated with complement-free antihuman rabbit's haemolytic serum and their resistance to haemolysis determined.

As a control test, a few experiments were made to determine the resistance of human erythrocytes after having been treated with normal fowl's serum in the same way as above.

In this way the resistance of the erythrocytes under different conditions was determined and the following tables worked out. The resistance of the erythrocytes is expressed in terms of the relative haemoglobin-value of the resistant erythrocytes which is the ratio between the amount of haemoglobin in the resistant corpuscles and the amount in the total suspension of erythrocytes used.

Tables shewing the resistance of erythrocytes to haemolysis under different conditions:—

TABLE I.

	Resistance of normal erythrocytes to haemolysis	Resistance of erythrocytes loaded with amboceptor obtained from fowl
1	0·345	0·500
2	0·379	0·456
3	0·242	0·407
4	0·333	0·480
5	0·346	0·452
6	0·269	0·531
7	0·333	0·466
8	0·350	0·444
9	0·301	0·421
10	0·288	0·459
11	0·288	0·368

TABLE II.

	Resistance of normal erythrocytes to haemolysis	Resistance of erythrocytes loaded with amboceptor obtained from rabbit
1	0·195	0·600
2	0·188	0·378
3	0·296	0·592
4	0·240	0·480
5	0·132	0·220

TABLE III.

	Resistance of normal erythrocytes to haemolysis	Resistance of erythrocytes treated with normal fowl's serum
1	0·288	0·243
2	0·288	0·298
3	0·282	0·259
4	0·240	0·282

It will be seen from the above tables that in every case the addition of the amboceptor increased the specific resistance of erythrocytes, while the addition of normal fowl's serum did not bring about any difference in their resisting power.

The problem that we are now to solve is how it is that the erythrocytes become more resistant when they become fixed to the amboceptor but lose all their resisting power as soon as the complement acts and what is the biological significance of this remarkable phenomenon. An explanation may be offered, which however is hypothetical. In the first process of the action of haemolysin during which the amboceptor combines with the erythrocytes, we may assume that this combination is purely of the nature of *adsorption* and not true chemical combination. In this process the dimensions of the pores between the molecules of the outer wall of the erythrocytes become small due to the amboceptor molecules filling the pores of the original erythrocytes. As diffusion depends, specially, upon the dimensions of the pores of the membrane through which it takes place, less haemoglobin passes out through the red corpuscles loaded with amboceptor than the unloaded ones when they are treated with distilled water. In the case of the colloidal complex of amboceptor and erythrocyte molecules, the bodies brought into close contact with each other do not react with one another in the chemical sense. Chemical reaction which, according to Bayliss [1912], is the third and last stage of the heterogeneous reactions of colloidal complexes, takes place between the molecules of the amboceptor and of the

erythrocytes only through the agency of the complement, which probably acts more or less like a ferment. When this takes place, there is a condensation of the molecules of the colloidal complex in each erythrocyte and as a result of this, the dimensions of the pores of the membranes of the red corpuscles increase to such an extent that haemoglobin diffuses out of the corpuscles and haemolysis results.

This theory will easily explain the inhibitory influence exerted by hypertonic saline solutions on the action of specific haemolysins as has been observed by Sutherland and McCay [1911] and others. Such saline solutions would tend to reduce the size of the erythrocytes by exosmosis of water from them and as a result of this the molecules of the erythrocytes come closer to each other and haemolysis is prevented. In other words the widening of the pores of the erythrocytes brought about by the action of the complement on the amboceptor-loaded erythrocytes is counteracted by the presence of the hypertonic saline.

To test the accuracy of the theory the following experiments were performed:—

A suspension of sheep's corpuscles in normal saline was mixed with amboceptor and complement and kept in the incubator. The process of haemolysis was stopped in from five to ten minutes before it was complete, the corpuscles quickly centrifuged and the supernatant fluid replaced by N/2 NaCl, after thoroughly washing the corpuscles several times with the same. A portion of the suspension of the corpuscles in N/2 NaCl was again centrifuged and the N/2 solution was pipetted off and in its place an excess of 0·85 % NaCl solution was substituted—a marked haemolysis resulted. Thus we have the following results:—

(1) Amboceptor-loaded corpuscles partially acted upon by complement, thoroughly washed in N/2 NaCl—no haemolysis.

(2) Amboceptor-loaded corpuscles partially acted upon by complement, thoroughly washed in N/2 NaCl and then the N/2 NaCl replaced by 0·85 % NaCl—distinct haemolysis.

In other words amboceptor-loaded corpuscles which have been partially acted upon by the complement are haemolysed when brought in contact with normal saline. The dimensions of the pores of such corpuscles when suspended in N/2 NaCl are much less than when suspended in normal saline, i.e. there is marked widening of these pores when they are suspended in normal saline and haemoglobin diffuses out. Evidently, therefore, the pores of the amboceptor-loaded corpuscles that have been acted upon partially by the complement are much wider than those of the normal ones. The

complement must have therefore brought about a condensation of the molecules of the amboceptor-loaded corpuscles, as explained before, thereby leading to widening of their pores to such an extent as to allow the haemoglobin molecules to pass out of them, even when they are suspended in normal saline.

An attempt was made to determine the volume of the corpuscles before and after combination with amboceptor. After rejecting different methods, the following method was devised for determining the size of the corpuscles. Human and sheep's corpuscles were used for the purpose.

The corpuscles were first suspended in 0·85 % NaCl and the suspension was sucked into two large-bore haematocrit tubes of exactly the same diameter and the corpuscles in both were centrifugalised at a high speed for about ten minutes, after which their volume was noted. The clear supernatant fluid from one of the tubes was now pipetted off and a dilution of haemolytic amboceptor in 0·85 % NaCl (one part of amboceptor and nine parts of 0·85 % NaCl) introduced in its place. The amboceptor was then thoroughly mixed with the corpuscles and the mixture allowed to stand for ten minutes. The other tube which was used as a control was then shaken up and the corpuscles again mixed with the supernatant fluid. The two tubes were again centrifugalised for ten minutes and the volume of the corpuscles noted. If the volume of the corpuscles in the control tube was found to be exactly equal to what was noted in it in the first experiment, then there was no error due to any change in the speed of the centrifuging tube. The volume of the amboceptor-loaded corpuscles was then noted. If the volume of the corpuscles in the control tube was different in the second experiment from what was noted in the first, then the volume of the amboceptor-loaded corpuscles was accordingly changed. In this way, absolutely accurate results were obtained.

Thus, if V be the volume of the corpuscles in the control tube after the first centrifuging and V' after the second, then $V - V'$ is the difference due to changes in the speed of the centrifuging machine.

If now v be the volume of the corpuscles before being loaded with amboceptor, and v' after being loaded with amboceptor, it is evident that $v - v'$ is not actually the change in the volume of the corpuscles. The exact change is, evidently,

$$(v - v') + \frac{v}{V}(V - V').$$

In this way, the exact change in the volume of the corpuscles due to combination with amboceptor was found and the following results obtained.

Human corpuscles.

Volume before action of amboceptor	Volume after action of amboceptor
(1) 2·75 cm.	(1) 2·75 cm.
(2) 2·75	(2) 2·75
(3) 2·50	(3) 2·50
(4) 1·50	(4) 1·50
(5) 1·45	(5) 1·50

Sheep's corpuscles.

Volume before action of amboceptor	Volume after action of amboceptor
(1) 3·30 cm.	(1) 3·30 cm.
(2) 5·38	(2) 5·20
(3) 4·50	(3) 4·20
(4) 6·23	(4) 6·22

It will be seen from the above tables that there was no reduction in the volume of the corpuscles after the action of the amboceptor and therefore the increase in their resistance to haemolysis was not due to any diminution in their volume after the action of the amboceptor.

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REFERENCES.

Bang (1910), *Biochem. Zeitsch.* **23**, 463.
Bayliss (1912), *Proc. Roy. Soc.* B, **84**, 81.
Brahmachari (1909), *Biochem. J.* **4**, 59, 280.
—— (1911), *Biochem. J.* **6**, 291. (See also *Studies in Haemolysis*, 2nd Ed.)
Landau (1903), *Ann. Inst. Past.* **17**, 52.
Sutherland and McCay (1911), *Biochem. J.* **5**, 1.

LIII. NOTES ON SOME FURTHER EXPERIMENTS ON THE CLOTTING OF CASEINOGEN SOLUTIONS.

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(*Received October 10th, 1913.*)

Some months ago, I communicated to the Royal Society the results of a series of experiments on the clotting of caseinogen solutions [1913]. It was there shown that caseinogen, when freshly precipitated from diluted skimmed-milk under certain specified precautions, is an unstable substance which on treatment with water at 37°, or with 0·1 % acetic acid at room temperature, is readily converted into another product or series of products (metacaseinogens), which differ from the original substance in that they are very much less soluble in lime water, and consist therefore apparently of smaller aggregates of lower molecular weight. When a half-saturated solution of lime water is saturated with the natural caseinogen by rotating the mixture for several hours in a thermostat at 20°, an acid milky solution is obtained after centrifugalisation and filtration, which readily clots without the addition of a soluble calcium salt in the presence of rennin; it will also clot in the absence of rennin when gently warmed (to 25°) in the presence of soluble calcium salts when the latter exist in the mixture within certain definite limits of concentration. In the presence of milk-serum, peptones, amino-acids etc., a clot can be produced from the above-mentioned calcium caseinogenate solution only in the presence of both soluble calcium salts and rennin.

In the paper quoted it was shown, furthermore, that the casein of the clot produced from calcium caseinogenate by rennin alone is about half as soluble in lime water as the natural caseinogen, and appears to possess, therefore, approximately half the molecular weight, and corresponds in properties with the metacaseinogens produced from natural caseinogen by the action of water.

Similar conclusions as to the molecular weight of casein, and its relations to caseinogen have been arrived at recently by Lucius van Slyke and A. W. Bosworth [1913], who have attacked the problem from a quite different

standpoint, and by other experimental methods, and in a still more recent paper by Bosworth [1913] a method is described for the production of a clot from caseinogen solutions by rennin alone, which does not differ materially from the method described by me[1].

Bosworth also came to the same conclusion as I did, that the production of casein from caseinogen is not an ordinary proteoclastic reaction, although it may be the result of a hydrolytic cleavage. I have ventured to suggest that the two substances bear a relationship to one another similar to that of metaphosphates to pyrophosphates. Bosworth and I are also in accord with reference to the identity, or at any rate the approximate identity, of the empirical chemical compositions of caseinogen and casein.

Accurate stœchiometrical relationships between casein and caseinogen were not to be attained by the method of experiment adopted by me, owing to the existence of an adsorption equilibrium when lime water is treated with excess of various caseinogen and casein preparations, a point which has been the subject of numerous experiments, which are briefly mentioned in my previous paper [1913, p. 466].

The casein prepared from the clot differs in one important respect from metacaseinogen produced by the action of water at 37° or by 0·1 % acetic acid at room temperature from caseinogen, for whereas the latter, on solution in excess of caustic alkali and reprecipitation from this solution with acetic acid under certain defined conditions is reconverted into a caseinogen of relatively high solubility in lime water, which readily yields clots on treatment either with calcium chloride or rennin, casein obtained by means of rennin cannot be converted into caseinogen under these conditions; on dissolving in alkali and reprecipitation, a product of only about half the solubility of caseinogen in half-saturated lime water is obtained. The solution in lime water furthermore does not clot on addition of rennin, and yields, on mixture with solutions of calcium salts within certain limits of concentration, an immediate precipitate. This precipitate is readily distinguishable from the clot produced under similar circumstances from caseinogenate solutions, which only forms when the temperature is raised above that of the temperature of solution.

Further experiments have been directed towards ascertaining the reason of this difference between the casein of the clot and the metacaseinogen

[1] Throughout my papers, I have consistently referred to the preparations which have not been clotted as caseinogen, whereas the preparations obtained from the clot are described as casein. In this respect, I have kept to the English method of nomenclature. Van Slyke and Bosworth adopt the method of nomenclature usual on the continent, describing the products as casein and paracasein, respectively.

produced by the action of water alone on caseinogen. These experiments are not yet complete, but I have been tempted to communicate them in their present form, owing to the fact that they have been subjected to a temporary interruption due to my change of laboratories.

Clotting of caseinogen solutions by means of pancreatin.

Investigations have been carried out with the object of comparing the process of clot production by means of other ferments. The results obtained with pancreatin indicate that its action can be readily distinguished from that of pepsin (or rennin). When half-saturated lime water is saturated with natural caseinogen, the solution thus obtained in the manner described [Schryver, 1913, p. 462] readily clots on the addition of small amounts of pepsin solution (1 %). *No soluble calcium salt need be added.* A clot under similar conditions cannot be produced by a pancreatin solution, although the latter will clot milk. A soluble calcium salt appears, therefore, to be necessary when pancreatin is employed for clot-production [compare Mellanby, 1912]. A clot was also prepared from an artificial milk by pancreatin in the following way: 5 c.c. of calcium caseinogenate solution were mixed with an equal bulk of milk-serum containing calcium chloride in the concentration of N/25. Under these conditions, as has already been shown, no clot is produced. If to this mixture are added a few drops of 1 % pancreatin solution, and the whole is incubated, a clot is produced similar in appearance to that produced by pepsin under like conditions.

The clot produced from artificial milk has the same properties as that produced from the natural milk, but differs in certain important respects from the clot produced by pepsin (or rennin) preparations.

The general method employed for examining the casein from the clots was as follows. The serum was poured off from the clot, which was then thrown on calico, and squeezed. It was then ground in a mortar with 0·1 % acetic acid, washed with water, dilute alcohol in increasingly graded strengths, absolute alcohol and ether, and then air-dried. The fine powder thus produced was then heated for 5 minutes with absolute alcohol to destroy the enzyme, the hot alcohol was filtered off by means of a Buchner funnel and the powder then washed with alcohol and ether, and again air-dried. The powder was then transferred to a mortar, ground with water into a paste, and N sodium hydroxide solution was added with continual grinding, until nearly all dissolved, and the solution was alkaline to phenolphthalein. It was then diluted and filtered through paper-pulp on to a Buchner funnel. Dilute

acetic acid was then carefully added with constant stirring until precipitation was complete, the supernatant liquid was rapidly poured off, and the precipitate washed by decantation or on a filter with ice-cold water, alcohol in graded strengths up to absolute alcohol, and finally with ether, and it was then air-dried.

Sometimes the above process was modified in various details, e.g. the clot after washing with water was first dissolved in alkali and reprecipitated, and the product after drying in the usual manner treated with hot alcohol. In all cases, however, a similar product was obtained. When the clots produced by pepsin and pancreatin were compared, the same procedure was always employed. The clots produced by pepsin alone from calcium caseinogenate solution, or by the combined action of the ferment and calcium chloride and ferment in the presence of milk-serum, yielded caseins with the same properties.

Now whereas the original caseinogen preparations had a solubility in half-saturated lime water of about 35[1], and after solution in alkalis and reprecipitation a solubility of about 30, the solubility of the casein preparation from the pepsin clot was about 14–17 and that of the pancreatin clot about 6–8. Unlike metacaseinogen, therefore, caseins are not converted into the more soluble products by solution in alkali and reprecipitation with acids, even when all precautions for preventing subsequent change have been taken. Furthermore, a saturated solution of caseinogen in half-saturated lime water is opaque like milk, whereas that of pepsin-casein is opalescent, and that of pancreatin-casein is generally water-clear. The two latter yield immediate precipitates and not clots on treatment with calcium chloride and do not clot on treatment with rennin. There appears, therefore, to be a difference in the molecular weights of the caseins produced by pepsin and pancreatin, both of which are considerably lower than that of caseinogen, that of pepsin-casein being about one-half and that of pancreatin-casein about one-quarter of that of caseinogen. Again, attention must be called to the fact that exact stœchiometrical relationships are not to be expected from the method of experiment employed.

The differences between metacaseinogens and caseins.

As already repeatedly stated, the metacaseinogens differ from the caseins in that the latter are not reconverted into caseinogen on solution in alkali

[1] I.e. 5 c.c. of the solution after Kjeldahlisation required 35 c.c. N/10 sulphuric acid to neutralise the ammonia produced (see former paper).

and reprecipitation with acids, whereas the former are. In spite of the large number of experiments which have been carried out, I have not so far succeeded in producing from a casein a solution which clots on treatment with rennin.

Investigations have, therefore, been carried out with the object of ascertaining the reason of the differences of behaviour between these substances, which in other respects have similar properties.

Up to the present, no entirely satisfactory proof as to the actual nature of the difference has been found, but certain facts have been recently discovered which afford some indication.

It seems not improbable that the ferment forms a combination with its substrate, from which it cannot afterwards be separated, and that the properties of the latter are then so altered that it can no longer form the big aggregates of the character of caseinogen.

Experiments were therefore carried out with the object of ascertaining the properties of the substance produced, when ferments were allowed to act on caseinogen at low and high temperatures, i.e., under conditions under which the proteoclastic action of the ferment is excluded.

Pepsin was allowed to act on calcium caseinogenate for 15 minutes at 0°, the conditions of experiment being otherwise the same as those employed in clot production. No clot formed under these conditions. The free caseinogen was then precipitated with ice-cold dilute acetic acid and a little sodium chloride[1], and rapidly washed several times with ice-cold water, graded strengths of alcohol, absolute alcohol and ether. These operations were carried out as rapidly as possible. The powder after air drying was then heated for 5 minutes with boiling alcohol, dissolved in excess of alkali, from the solution in which the casein(ogen) was obtained in the usual way. Although the product thus obtained possessed about the same solubility in half-saturated lime water as ordinary caseinogen which had been dissolved in alkali and reprecipitated, the solution obtained from caseinogen which had been treated with the pepsin differed from that got from normal caseinogen (treated in a similar way but without pepsin), in that the former did not clot in the presence of rennin, and yielded an immediate precipitate, and not a clot, when treated with calcium chloride solution of the requisite concentration.

The mere precipitation therefore, of caseinogen in the presence of a ferment under conditions which exclude enzyme action, causes appreciable alterations in its properties. When one considers the important part that surface

[1] The precipitate does not readily separate in ice-cold solutions without this addition.

actions play in such processes, this result is not altogether surprising. The above result was obtained several times. A similar experiment was also carried out in which pancreatin was used instead of pepsin, the conditions being the same as those described above. The product in this case had a solubility of only about 8 in half-saturated lime water, and was identical in properties with the casein obtained from a pancreatin clot. It may be remarked here that pancreatin produces the clot in the highly acid solution.

A large number of experiments were also carried out with the object of ascertaining the action of the heated ferment on clot formation. The results obtained up till now are not, however, quite conclusive, and this is due to the varying properties of the different pepsin preparations. Rohonyi [1913] has recently succeeded in preparing definite compounds of proteins with proteoclastic ferments, and has shown that such compounds can be obtained when ferment solutions are employed, the proteoclastic activity of which has been destroyed by heat. If the conception is true that the caseins differ from metacaseinogens in the fact that the former are combinations with the ferments, then, in the light of Rohonyi's recent work, it is conceivable that caseins should be formed by the action of heated enzymes. In carrying out the experiments devised for testing this theory precisely the same difficulties have been encountered as those described by Rohonyi, due to the differences of characters of the various enzyme preparations. This investigator has shown that it is possible to prepare compounds of pepsin and proteins by the employment of solutions of the ferment which have been heated for 15 minutes at 100° *provided that no precipitate is formed during the heating.* Preparations having this property appear to be rare. I have myself not succeeded in obtaining such. I have, however, obtained a preparation which after heating to 80° for some time was still capable of actively clotting calcium caseinogenate solutions. Prolonged heating of the ferment solution in quartz tubes at this temperature produced a precipitate, and the solution gradually became inactive.

It is very probable that experiments of this nature will only succeed when a ferment preparation relatively free from extraneous proteins is obtained. Further work in this direction is contemplated.

The nature of "natural caseinogen."

L. van Slyke and Bosworth have called attention to the fact that various commercial preparations of caseinogen contain large quantities of acid calcium salt, due apparently to the precipitation of the product with insufficient quantities of acid. A criticism might therefore be directed against the "natural caseinogen" described by me, that such consisted of a mixture of free acid and acid calcium salt. I therefore subjected my preparation to examination with a view of ascertaining its nature, and comparing it with a calcium-free preparation prepared according to the method of van Slyke and Bosworth. The latter was found to have a low solubility in half-saturated calcium hydroxide, as it had stood some time in contact with hydrochloric acid during the process of filtration. After solution in alkali, and reprecipitation with acid, with the precautions already repeatedly described, it had a solubility of about 26, i.e., about the solubility of my own caseinogen preparations which have been "purified" by solution in alkali and reprecipitation by acid[1].

The solubilities of my "natural caseinogen" and of the caseinogen preparations prepared according to van Slyke and Bosworth (both the preparation directly precipitated with hydrochloric acid and that redissolved in alkali and reprecipitated by acetic acid with precautions for preventing change) in warm 10% sodium chloride were determined. Van Slyke and Bosworth show that under these conditions the calcium salts readily dissolve, whereas the free acids do not. The preparations were mixed with 10 times their weight of the saline solution and maintained at 37° for about 1 hour with continual shaking. The mixtures were afterwards centrifuged, and the nitrogen in the clear filtered supernatant liquid was determined by Kjeldahl's process.

The saline extract from all preparations contained about the same small amount of nitrogen, viz. 2 or 3 mgrms. in 5 c.c. Only minute quantities of the preparations were soluble in saline, and my "natural caseinogen" is therefore a free acid and not a calcium salt. As a control, an acid calcium salt was prepared by precipitation of sodium caseinogenate solution by calcium chloride [Schryver, 1913, p. 469]. The precipitate was washed with dilute alcohol till chlorine free, and obtained dry after alcohol and ether washing in the usual way. On treating this with 10 times its weight of 10% sodium chloride at 37°, it rapidly dissolved, leaving only a very small residue, and yielding a thick syrupy solution.

[1] Preparations thus "purified" have never as high a solubility in lime water as "natural caseinogen."

Conclusions.

1. Casein produced from a pepsin (or rennin) clot differs from metacaseinogen, a product produced by the action of water at 37° on caseinogen, in that it cannot be converted by solution in alkali and reprecipitation into a more soluble product which dissolves in calcium hydroxide to yield clottable solutions. The statement made in my earlier paper that casein produced by pepsin is an aggregate of about half the size of that of caseinogen is confirmed.

2. The action of pancreatin in clot-production differs from that of pepsin, in that it produces clots only in the presence of soluble calcium salts. It produces these clots, however, under conditions under which calcium salts alone fail, e.g., in the presence of milk-serum. The casein from pancreatin clots also differs from that of pepsin clots. The former have only about half the solubility in half-saturated lime water of the latter. Such lime water solutions are usually water-clear.

3. Certain experiments are quoted, which indicate that the difference between metacaseinogens and caseins is due to the fact that the latter are combinations of the enzyme with the protein. The evidence produced both by myself and independently by van Slyke and Bosworth and by Bosworth alone, indicate that the action of the ferment is not an ordinary proteoclastic action. It is conceivable that if the enzyme contains both haptophoric and zymophoric groups, the former only take part in clot formation.

4. Evidence is brought to show that the "natural caseinogen," the preparation of which is described in my earlier paper, is not a calcium salt.

References.

Bosworth (1913), *J. Biol. Chem.* **15**, 231.
Mellanby (1912), *J. Physiol.* **45**, 345.
Rohonyi (1913), *Biochem. Zeitsch.* **53**, 179.
Schryver (1913), *Proc. Roy. Soc.* B. **86**, 460.
Van Slyke, L. and Bosworth, A. W. (1913), *J. Biol. Chem.* **14**, 203, 207, 211, 227 and 231.

LIV. ON THE CHOLESTEROL CONTENT OF THE TISSUES OF CATS UNDER VARIOUS DIETETIC CONDITIONS AND DURING INANITION.

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Since the discovery of cholesterol by Conradi in 1775, and its analysis by Chevreul in 1815, it has been found to be very widely distributed in the animal, and in its isomeric forms in the vegetable, kingdom, and it is now generally recognised as an integral constituent of all cells in the animal body [Dorée, 1909]. The constant presence of such a substance clearly suggests its importance from a vital standpoint, and necessitates its recognition as a primary constituent of all protoplasm. Though a considerable amount of work has been done on the subject, however, we have as yet little definite knowledge of its physiological functions.

As a result of experiments on herbivorous animals published by one of the present writers and various colleagues and the researches of Pribram [1906], Kosumoto [1908, 1 and 2], Harley and Barratt [1903] the working hypothesis was put forward that in these animals cholesterol is a substance which is strictly conserved in the animal organism; that when the destruction of the red blood corpuscles and possibly other cells takes place in the liver, their cholesterol is excreted in the bile, and that the cholesterol of the bile is reabsorbed in the intestine along with the bile salts and finds its way into the blood stream to be used in cell anabolism [Dorée, Ellis, Fraser and Gardner, 1908–1912]. In the case of carnivorous animals there would not appear to be the same necessity for such economy, as cholesterol is a normal constituent of their food, whereas the food of herbivora contains no cholesterol, but instead the isomeric phytosterols, and these only in minute quantity. The absorption of the food cholesterol would, however, be limited by the reduction of cholesterol to coprosterol by the bacteria of the gut. In the

human subject cholesterol is excreted in the faeces entirely in the form of coprosterol under ordinary conditions [Bondzynski, 1896; Bondzynski and v. Humnicki, 1896], though it has been shown by Müller [1900] that a prolonged milk diet leads eventually to the excretion of cholesterol as such. In the case of dogs and cats the change into coprosterol is generally only partial, though under some conditions it may be complete, and takes place only on meat diets [Dorée and Gardner, 1908, 2]. Feeding experiments [Dorée and Gardner, 1908, 2; Kosumoto, 1908, 2; Ellis and Gardner, 1909, 2] on dogs and cats have shown that the total cholesterol found in the faeces, either as cholesterol or coprosterol can be more than accounted for by the cholesterol taken in with the food, provided that the animal remains in health and constant in weight. This is also probably the case in man [Ellis and Gardner, 1912, 3]. Feeding cats, however, on artificial diets free from cholesterol led to inconclusive results from the point of view of deciding whether in the case of carnivora the cholesterol of the bile is normally reabsorbed along with the bile salts in the intestine [Ellis and Gardner, 1909, 2]. Earlier experiments made by Dorée and Gardner [1909] to trace a connexion between the percentage of cholesterol in the blood of dogs and the cholesterol content of the food taken, also led to inconclusive results, partly owing to the defects in the methods then available for estimating cholesterol in the tissues. Recent improvements in the methods of estimating cholesterol—notably the digitonine process of Windaus [1910]—rendered it desirable to repeat and extend the experiments referred to. In the present paper we give an account of analyses of blood and the tissues of cats fed on diets containing different amounts of cholesterol and also of cats kept in a state of inanition.

It is much more difficult in the case of carnivorous animals to maintain constant conditions during experiment than in the case of herbivorous animals such as the rabbit. In the latter animals a natural standard diet, free from cholesterol and similar bodies, to which measured portions of cholesterol could be added was available; and as these animals are practically continuous feeders the bile flow and consequently the cholesterol content of the blood due to this source would remain practically constant. In the case of cats we had to make use of different foods, though the difference was not so marked as in the earlier experiments. Little is known concerning the influence of food on the secretion of the bile, but from experiments that have been made by various observers, there is good reason to suppose that the nature of the diet would not be without influence [*vide* Goodman, 1907]. Furthermore the cat and the dog are discontinuous feeders and the flow

of bile into the intestine would be intermittent. Under these circumstances the portion of the floating as distinguished from the constituent cholesterol in the blood and the tissues due to the reabsorption of the cholesterol of the bile, would not necessarily be strictly comparable in different cases. What would be the limits of such variation, if any, we have no data at present upon which to form an opinion, but a variation of the kind suggested might wholly or partially mask any variation due to the cholesterol absorbed from the food, which at best would not be great in absolute magnitude. We did not therefore expect to obtain results comparable in precision with those obtained in the case of herbivora, but we attempted to render the conditions as comparable as possible by caréful selection of diets, and by always killing the animals during a period of active digestion.

Details of the diets used.

Diet A, cholesterol free. We had some difficulty in finding a cholesterol-free diet which cats would take freely, but ultimately the following proved suitable. The whites of four eggs were taken raw and mixed with an equal weight of mashed boiled potatoes. The well-mixed material was flavoured with a small quantity of Liebig's extract of meat, and heated until the white of egg coagulated. The animals appeared to relish this diet, not the slightest trouble being experienced in getting them to eat it.

Diet B. Diet A, with the addition to each daily ration of a weighed amount of free cholesterol.

Diet C. Lean cooked beef.

Details of the animals used.

Diet A, cholesterol free.

Cat No. 1. The animal was fed for eight days and consumed 1003 grams white of egg and 1003 g. potatoes, the total weight when cooked being 1932 g. During this period its weight remained quite constant—2·7 kilos. It was killed one hour after the last meal. The stomach was full, and all the organs appeared normal.

Cat No. 2. This cat ate the same amount of food as No. 1, and during the eight days of the diet period its weight was as follows:—2·67, 2·58, 2·6, 2·55, 2·5, 2·5 and 2·55 kilos. It was killed two hours after the last meal. The stomach was full, and all the organs seemed normal.

Cat No. 3. This cat was pregnant, and during 11 days consumed

728 g. of egg white, 728 g. of potatoes, weighing when cooked 1396 g. It lost weight gradually during the diet period—3, 2·7, 2·7, 2·5, 2·4, 2·3 and 2·3 kilos. It was killed one hour after the last meal.

Cat No. 4. This animal ate in the course of 11 days 1138 g. egg white and 1210 g. of potatoes, weighing when cooked 2188 g. It remained approximately constant in weight—3·2, 3·2, 3·2, 3·1, 3·0, 3·1, 3·1, 3·1 kilos. It was killed *three* hours after the last meal. The stomach was half full, and the organs seemed normal.

Diet B.

Cat No. 5. During 11 days this cat consumed 693 g. of egg white and 693 g. of potatoes, weighing when cooked 1256 g., and 2·75 g. of cholesterol, i.e. about twice the amount it would have got from the same weight of meat. It weighed 2·3 kilos. at the beginning of the experiment and 2·2 kilos. at the end. It was killed one hour after the last meal, and all its organs were quite normal.

Cat No. 6. This cat consumed during 11 days 728 g. of egg white and 728 g. of potatoes, weighing when cooked 1303 g., and 2·75 g. of cholesterol. It weighed 2·4 kilos. at the beginning and gradually decreased in weight to 2·1 kilos. It was killed 1·5 hour after the last meal. Its organs seemed normal.

Diet C.

Cat No. 7. This animal was fed for ten days on lean cooked beef as free as possible from fat and consumed 1563 g. of meat weighing in the raw state 2165 g. This would correspond to an intake of about 1·7 g. of cholesterol. The weights taken from time to time were 3·37, 3·3, 3·4, 3·33, 3·42, 3·45 and finally 3·43 kilos. It was killed four hours after the last meal. The stomach was full and all the organs normal.

Cat No. 8. This cat consumed during 11 days 1910 g. of cooked lean beef, weighing in raw state 2433 g. This would correspond to an intake of about 1·9 g. of cholesterol during the period. The animal remained constant in weight—3·3 kilos. It was killed four hours after the last meal. The stomach was full and the organs normal.

Animals in a state of inanition.

In these experiments well-fed animals were kept without food for seven days, but were allowed as much water as they wished. At the end of this period they all appeared to be in good health. No faeces were passed.

Cat No. 9. The weights taken daily, except on Sunday, were 3·1, 2·9, 2·8, 2·6, 2·55 and 2·5 kilos. The loss of weight was therefore 19·4 %. After the animal was killed the stomach was found to be completely empty. There was a little fluid in the small intestine, and some faecal matter in the rectum.

Cat No. 10. The weights recorded were 3·1, 2·95, 2·8, 2·75, 2·7, 2·6 and 2·5 kilos., the loss in weight being as before 19·4 %. The stomach was quite empty; a small amount of fluid was found in the small intestine and some faeces in the rectum.

Cat No. 11. This animal similarly treated only lost 10 % of its body weight, the weights recorded from time to time being 2·8, 2·7, 2·6, 2·5 and finally 2·5 kilos. The stomach was empty, but there was some fluid in the intestine and faeces in the rectum. The weight of the contents was 92 g., and this was found to contain 0·24 g. of cholesterol.

Immediately the animals were killed, they were cut up and the various tissues submitted with the least possible delay to the treatment described below. The following were examined—blood, liver, muscle taken from various parts of the body, kidney, suprarenal glands, heart, brain, lung and contents of the gall-bladder.

Method of extraction and estimation of cholesterol.

The tissues were finely minced and ground up with sand and enough plaster of Paris to make the whole mass fairly dry and friable. It was allowed to stand until quite dry, powdered and extracted in a Soxhlet's apparatus with ether for about 14 days. The ethereal extract was made up to known volume, and divided into two portions, or in some cases into suitable aliquot portions. In one portion the free cholesterol was estimated and in the other the total free and ester cholesterol after saponification in ethereal solution by means of sodium ethylate. The cholesterol was estimated by the modification of Windaus' digitonine process described by Fraser and Gardner [1910]. The ester cholesterol was thus determined by difference, and it was assumed that no hydrolysis of the esters took place in the time occupied in dissecting out the tissue, mincing up and drying with plaster of Paris. Though we think this assumption is probably justifiable in the case of most of the tissues, it is perhaps open to some question in the case of liver. This tissue is known to contain ferments, which hydrolyse cholesterol esters, but the hydrolysis does not appear to take place rapidly even under most favourable conditions. Thus a sample of liver which on treatment as

described above was found to contain 0·28 % of total cholesterol and 0·13 % of free cholesterol and therefore 0·15 % of ester cholesterol, was still found to contain 0·05 % of ester after incubating for four days at 37° in saline suspension in the presence of a little toluene, though another sample allowed to putrify in saline suspension at 37° yielded no ester. We endeavoured however to reduce any such error to a minimum by carrying out the operations as rapidly as possible.

Discussion of analytical results.

Blood. The results of the analysis of the blood of the eleven animals are collected together in Table I.

TABLE I.

No. of cat	Diet	Weight of blood taken for analysis, in grams	Total free and combined cholesterol in g. per 100 g. of blood	Free cholesterol in g. per 100 g. of blood	Cholesterol in form of esters per 100 g. of blood
1	A (non chol.)	68·2	0·044	0·044	Nil
2	A	87·9	0·060	0·046	0·014
3	A	84·1	0·058	0·056	0·002
4	A	83·2	0·044	0·040	0·004
		Mean of 4 cats	**0·052**	**0·047**	**0·005**
5	B (cholest.)	74·2	0·102	0·098	0·004
6	B	59·8	0·178	0·049	0·129
		Mean of 2 cats	**0·140**	**0·074**	**0·066**
7	C (meat)	126·2	0·083	0·066	0·017
8	C	74·2	0·078	0·054	0·024
		Mean of 2 cats	**0·081**	**0·060**	**0·021**
9	Inanition	65·8	0·163	0·074	0·089
10	Inanition	76·5	0·196	0·069	0·127
11	Inanition	75·6	0·151	0·126	0·025
		Mean of 3 cats	**0·170**	**0·090**	**0·080**

On comparing the figures in column four of Table I it will be seen that the total cholesterol of the blood increases with the cholesterol taken in with the food. In the case of the animals in a state of inanition, which were living on their own tissue, the increase is very marked. This increase is in all cases chiefly due to an increased quantity of ester cholesterol, though the free cholesterol itself also increases. The high ester content is very marked in the case of animals in a state of inanition. This may very likely have

something to do with the transference of the fat from the depots during starvation, and in this connexion it may be noted that in the case of cats 9 and 10 which lost 19 % of their body weight their blood contains 0·089 and 0·127 % of ester cholesterol, while that of cat 11, which only lost 10 % of body weight, is 0·025. In the case of cats 2 and 3, both of which lost weight during the diet period, the total cholesterol of the blood is somewhat higher than in the case of cats 1 and 4 which had the same diet but remained constant in weight. The same thing will be noticed in the case of cat 6 compared with cat 5 on the same diet.

The variations observed are similar to those found in the case of rabbits fed on diets containing varying amounts of cholesterol, and the figures are of much the same order as will be seen from Table II in which only average values are given.

TABLE II.

Number of animals of which the figures are the average	Animal	Diet	Total free and combined cholesterol per cent.	Free cholesterol per cent.	Ester cholesterol per cent.
4	Cat	Cholesterol-free A ...	0·052	0·047	0·005
4	Rabbit[1]	Bran extracted by ether ...	0·058	0·044	0·014
2	Cat	B (A + cholesterol) ...	0·140	0·074	0·066
4	Rabbit[1]	Extracted bran + cholesterol	0·081	0·059	0·022
2	Cat	C (lean beef)	0·081	0·060	0·021
2	Rabbit[1]	Ordinary bran	0·082	0·069	0·013
2	Rabbit[1]	Cabbage leaves and stalk ...	0·068	0·063	0·005
3	Cat	Inanition	0·170	0·090	0·080
2	Rabbit[1]	Inanition	0·154	0·113	0·040

[1] [Ellis and Gardner, 1912, 2.]

Liver. In Table III we give the total cholesterol, free and combined, actually found in the livers of the animals, also the weights of cholesterol per cent. of liver and the weights of liver cholesterol per kilo. of body weight. On comparing the figures for animals fed on diet A (cholesterol free) it will be seen that both the total percentages of cholesterol in the liver and the total liver cholesterol per kilo. of body weight are fairly uniform, though not so constant as was found to be the case in rabbits fed on cholesterol-free diet. On comparing these figures with those of cats 5 and 6 which were fed for a period on the same diet, but with the addition of free cholesterol, a marked increase in the total cholesterol is obvious. This increase is partly due to free cholesterol, but mainly to ester cholesterol. On the other hand in the case of cats 7 and 8, fed on lean meat, i.e. a diet containing

TABLE III.

No. of cat	Diet	Wt. of animal when killed, in kilos.	Wt. of liver, in g.	Wt. of total cholesterol in liver, in g.	Wt. of free cholesterol in liver, in g.	Wt. of ester cholesterol in liver, in g.	Wt. of cholesterol per cent. of liver			Wt. of liver cholesterol per kilo. of body wt.		
							Total	Free	Ester	Total	Free	Ester
1	A	2·70	59·8	0·128	0·092	0·036	0·214	0·154	0·060	0·047	0·034	0·013
2	A	2·55	54·6	0·136	0·041	0·095	0·249	0·076	0·173	0·053	0·016	0·037
3	A	2·30	63·0	0·102	0·102	Nil	0·162	0·162	Nil	0·044	0·044	Nil
4	A	3·10	70·8	0·192	0·048	0·144	0·254	0·067	0·187	0·062	0·015	0·047
					Mean values		**0·220**	**0·115**	**0·105**	**0·052**	**0·027**	**0·025**
5	B	2·20	48·4	0·194	0·089	0·105	0·401	0·185	0·216	0·088	0·041	0·047
6	B	2·10	43·7	0·228	0·049	0·179	0·466	0·117	0·349	0·108	0·023	0·085
					Mean values		**0·434**	**0·151**	**0·283**	**0·098**	**0·032**	**0·066**
7	C	3·43	77·5	0·243	0·221	0·022	0·316	0·285	0·031	0·071	0·064	0·007
8	C	3·30	107·7	0·362	0·362	Nil	0·346	0·346	Nil	0·109	0·109	Nil
					Mean values		**0·331**	**0·316**	**0·015**	**0·090**	**0·087**	**0·003**
9	Inanition	2·50	50·3	0·345	0·063	0·282	0·550	0·125	0·425	0·138	0·025	0·113
10	Inanition	2·50	49·8	0·295	0·069	0·226	0·637	0·143	0·494	0·118	0·027	0·091
11	Inanition	2·50	48·2	0·245	0·059	0·186	0·509	0·122	0·387	0·098	0·024	0·074
					Mean values		**0·565**	**0·130**	**0·435**	**0·118**	**0·025**	**0·093**

a considerable proportion of cholesterol but no added carbohydrate, there is a similar increase in total cholesterol, but the increase is almost entirely free cholesterol, the ester being nil in one case and considerably below the average of diet A in the other. In the case of cats 9, 10 and 11, in which the animals were living on their own tissues, we find a similar storing up of the cholesterol in the liver. It is stored almost entirely in the form of esters and there is only a slight increase if any of free cholesterol. This may also have something to do with the transference of the fat from the depots to the liver during starvation. In the case of cat 11, which lost only 10% of body weight, this storing up is not quite so great; in this cat the percentage in the blood was also somewhat lower than in the case of cats 9 and 10. Those changes are generally similar to those observed by Ellis and Gardner [1912, 1] in the case of rabbits during starvation. Assuming that the loss in weight of the cats during inanition is due to muscle tissue, then, if the cycle postulated for herbivora be true for carnivora, cats 9 and 10 both of which lost 600 g. of body weight should have accumulated each about 0·5 g. of cholesterol in the blood, liver and the contents of the intestine. Taking the average figures for diet A as representing the normal cholesterol content of the blood and liver under conditions in which body weight is kept constant, but no cholesterol is absorbed with the food, 0·42 g. of cholesterol was accumulated in the blood and liver of cat 9 and 0·41 in cat 10. The

contents of their intestines were not analysed, but in cat 11 about 0·2 g. was found.

Suprarenal Glands. This organ was only examined in five of the eleven cats, and the results are given in Table IV.

TABLE IV.

No. of cat	Diet	Weight of organ, in g.	Total cholesterol, free and combined, actually found	Free cholesterol actually found	% of total cholest.	% of free cholest.	% of ester cholest.
3	A	0·6569	0·0116	0·0060	1·77	0·91	0·86
4	A	0·5886	0·0155	0·0034	2·63	0·58	2·05
5	B	0·6310	0·0373	0·0373	5·92	5·92	Nil
6	B	0·4408	0·0228	0·0070	5·13	1·59	3·58
11	Inanition	0·5208	0·0048	0·0035	0·92	0·67	0·25

Here the addition of cholesterol to the diet produces a marked increase in the total cholesterol content of the organ, and the change appears to run parallel to that in the blood. In cat 5, for instance, the increase in the blood is all free cholesterol, the actual amount of ester cholesterol being slightly below the average of the cats on diet A. In the suprarenal the increase is also all free cholesterol, the ester being nil or very small. In cat 6 on the other hand, where the increase is almost entirely in ester cholesterol, the ester cholesterol of the blood is very high, while the free cholesterol is almost exactly equal to the average on diet A. In inanition however this parallelism no longer appears to hold, for while the cholesterol accumulates in the blood it decreases almost to the vanishing point in the suprarenals. More experiments will however be required before this interesting point can be settled. The inanition result appears, however, to be in agreement with the observations of Landau [1913]. In this connexion it may also be noted that Albrecht and Weltmann [1911] and Hueck [1911] have shown that in diseases such as Carcinoma, Tuberculosis, etc., both the free and ester cholesterin of the suprarenals sink and sometimes are absent altogether.

Muscle. The muscle analysed was a mixed sample cut from various parts of the body. The results are given in Table V.

TABLE V.

No. of cat	Diet	Percentage total cholesterol	Percentage free cholesterol	Percentage ester cholesterol
1	A	0·056	0·031	0·025
2	A	0·047	0·032	0·015
7	C	0·087	0·083	0·004
8	C	0·081	0·073	0·008
9	Inanition	0·059	0·053	0·006
10	Inanition	0·065	0·051	0·014

It will be noticed that on diet A, which contained no cholesterol, but was rich in carbohydrate, there is a marked increase in the ester cholesterol compared with the cats on meat diet C. The free cholesterol is however much lower. In the case of rabbits, Ellis and Gardner were unable to trace any influence of food cholesterol on the cholesterol content of muscle.

Heart. The results are given in Table VI.

TABLE VI.

No. of cat	Diet	Wt. of organ, in g.	Total cholest. per cent.	Free cholest. per cent.	Ester cholest. per cent.
1	A	7·9	0·080	0·073	0·007
2	A	8·8	0·068	0·068	Nil
7	C	16·3	0·073	0·073	Nil
8	C	12·5	0·072	0·063	0·009
9	Inanition	10·5	0·071	0·063	0·008
10	Inanition	9·4	0·091	0·039	0·052

The cholesterol of this organ is very constant, and nearly all present in the free state. The percentage is much the same as that given for heart muscle in the ox, 0·066 to 0·071 by Ellis and Gardner [1908].

Kidney. The results on this organ are given in Table VII.

TABLE VII.

No. of cat	Diet	Wt. of animal when killed, in kilos.	Wt. of kidney, in g.	Total cholesterol, in g.	Free cholesterol, in g.	Ester cholesterol, in g.	Wt. of cholesterol per cent. of kidney			Wt. of kidney cholesterol per kilo. of body wt.		
							Total	Free	Ester	Total	Free	Ester
1	A	2·7	21·6	0·064	0·064	Nil	0·298	0·296	0·002	0·024	0·024	Nil
2	A	2·6	17·8	0·056	0·056	Nil	0·326	0·326	Nil	0·022	0·022	Nil
7	C	3·43	27·5	0·073	0·069	0·004	0·265	0·252	0·013	0·021	0·020	0·001
8	C	3·3	24·8	0·077	0·070	0·007	0·313	0·284	0·029	0·023	0·021	0·002
						Mean values	**0·301**	**0·290**	**0·011**			
9	Inanition	2·5	20·8	0·070	0·053	0·017	0·335	0·212	0·123	0·028	0·021	0·007
10	Inanition	2·5	23·8	0·066	0·054	0·012	0·275	0·226	0·049	0·026	0·022	0·004

The diet as in the case of rabbits appears to have no influence on the kidney cholesterol. The average values for normal animals are not very dissimilar from the mean of those given by Windaus [1910] for normal human kidneys, viz. 0·24 free and 0·02 ester per cent. In the case of the starved animals the ester cholesterol shows a marked increase, which Ellis and Gardner also showed to be the case in the rabbit. These high ester values recall the high ester value found by Windaus in human pathological kidneys.

Lung. The results are given in Table VIII.

TABLE VIII.

No. of cat	Diet	Wt. of organ taken for analysis, in g.	Percentages of cholesterol		
			Total	Free	Ester
1	A	17·1	0·416	0·295	0·121
2	A	15·6	0·361	0·296	0·065
7	C	17·5	0·435	0·247	0·188
8	C	11·4	0·453	0·270	0·183
9	Inanition	13·4	0·445	0·413	0·032
10	Inanition	16·5	0·376	0·376	Nil

The cholesterol content, as one would expect, appears to have nothing to do with diet. The values are of much the same order as those given for rabbits by Ellis and Gardner, viz. average of six animals, free 0·442 and ester 0·053. The ester cholesterol, as in the case of rabbits, is very variable.

Brain. The brains of cats 1 and 2 on diet A, 7 and 8 on diet C and 9 and 10 in a state of inanition, were examined with the following results:— 3·05, 2·65, 2·94, 2·91, 2·33 and 2·06 per cent. In no case was any ester found. The average is 2·66. For rabbits, Ellis and Gardner give as an average of eight experiments 2·32, with a variation of 2·02–2·88, and in no case was there any evidence of the presence of esters.

Bile. Attempts were made to estimate the cholesterol content of the bile of the cats under different diets and during starvation, but the contents of the gall-bladder were so small that it was difficult to estimate the cholesterol with any degree of accuracy. We give however the results for what they are worth in the following Table IX.

TABLE IX.

No. of cat	Diet	Wt. of bile in gall-bladder, in g.	Percentage of cholesterol		
			Total	Free	Ester
1	A	0·96	0·56	0·42	0·14
2	A	1·48	0·28	0·28	Nil
7	C	0·61	0·36	0·34	0·02
8	C	1·30	0·61	0·61	Nil
9	Inanition	2·53	0·25	0·03	0·22
10	Inanition	1·62	3·01	3·01	Nil

The results of the experiments on diets A and C do not indicate any connexion between either the actual amount of cholesterol in the bile or the percentage content and the diet. In the case of cat 10 there was a relatively high percentage of cholesterol in the bile, and this appeared to be all or mainly in the free state. This is in agreement with the observations of Ellis and Gardner on starving rabbits. On the other hand in the case of cat 9, also in a state of starvation, both the actual amount found and the percentage are of much the same order as in the fed animals, but it was nearly all found in the ester condition.

General Conclusions.

The results obtained in these experiments are strikingly similar to those obtained by Ellis and Gardner on the rabbit, and are in agreement with the hypothesis advanced at the beginning of this paper, viz. that cholesterol is a constituent constantly present in all cells, and that when these cells are broken down in the life process the cholesterol is not excreted as a waste product, but is utilised in the formation of new cells. A function of the liver is to break down dead cells, e.g. blood corpuscles, and eliminate their cholesterol in the bile. After the bile has been passed into the intestine in the process of digestion, the cholesterol is reabsorbed, possibly in the form of esters, along with the bile salts and is carried in the blood stream to the various centres and tissues for reincorporation into the constitution of new cells. Waste of cholesterol is made up from that taken in with the food.

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REFERENCES.

Albrecht and Weltmann (1911), *Wiener med. Wochensch.* No. 14.
Bondzynski (1896), *Ber.* **29**, 276.
—— and v. Humnicki (1896), *Zeitsch. physiol. Chem.* **22**, 396.
Dorée (1909), *Biochem. J.* **4**, 72.
Dorée and Gardner (1908, 1), *Proc. Roy. Soc.* B, **80**, 212.
—— —— (1908, 2), *Proc. Roy. Soc.* B, **80**, 227.
—— —— (1909), *Proc. Roy. Soc.* B, **81**, 109.
Ellis and Gardner (1908), *J. Physiol.* **38**, *Proc.* i.
—— —— (1909, 1), *Proc. Roy. Soc.* B, **81**, 129.
—— —— (1909, 2), *Proc. Roy. Soc.* B, **81**, 505.
—— —— (1912, 1), *Proc. Roy. Soc.* B, **84**, 461.
—— —— (1912, 2), *Proc. Roy. Soc.* B, **85**, 385.
—— —— (1912, 3), *Proc. Roy. Soc.* B, **86**, 13.
Fraser and Gardner (1909), *Proc. Roy. Soc.* B, **81**, 230.
—— —— (1910), *Proc. Roy. Soc.* B, **82**, 559.
Goodman (1907), *Beiträge*, **9**, 91.
Grigaut (1913), *Le Cycle de la Cholestérinémie.*
Harley and Barratt (1903), *J. Physiol.* **29**, 341.
Hueck (1911), *Münchener med. Wochensch.* 2588.
Kosumoto (1908, 1), *Biochem. Zeitsch.* **13**, 354.
—— (1908, 2), *Biochem. Zeitsch.* **14**, 416.
Landau (1913), *Deut. med. Wochensch.* **12**, 1.
Müller (1900), *Zeitsch. physiol. Chem.* **29**, 129.
Pribram (1906), *Biochem. Zeitsch.* **1**, 414.
Wacker (1912), *Zeitsch. physiol. Chem.* **80**, 381.
Windaus (1909), *Ber.* **42**, 238.
—— (1910), *Zeitsch. physiol. Chem.* **65**, 110.

LV. ON THE OXIDATION OF COPROSTEROL AND COPROSTANONE.

PART I.

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Dorée and Gardner [1908] showed that coprosterol, $C_{27}H_{48}O$, was readily oxidised on treatment with the theoretical quantity of chromic acid with the production of the corresponding ketone, coprostanone, $C_{27}H_{46}O$. This coprostanone crystallised in glistening leaves which, under the microscope, appeared to consist of thin plates, generally square, with one or all of the corners slightly truncated. It melted at 62–63° to a clear liquid. It yielded an amorphous semicarbazone, melting at 192°, and an amorphous oxime, melting at 71°. On treatment with phenylhydrazine it behaved in an abnormal manner and yielded a crystalline derivative, melting at 192°, which was subsequently shown by Dorée [1909] to be coprosteryl-carbazole, formed from the coprostanone-phenylhydrazone with elimination of ammonia. As the yield of pure coprostanone in the above preparation was only 60%, it appeared desirable to submit the reaction to further investigation.

Oxidation of coprosterol by chromic acid. To make sure of oxidising the whole of the coprosterol, a considerable excess of chromic acid was used—three times the amount taken by Dorée and Gardner.

10 g. coprosterol dissolved in 130 cc. of glacial acetic acid, and heated to 70°, were treated in the course of 1 hour with a solution of 8·5 g. chromic anhydride in a little dilute acetic acid, the temperature being maintained by the heat of the reaction. After adding the chromic acid, the liquid was heated on the water bath for one hour, and then diluted with water. The bulk of the acetic acid was next neutralised with sodium carbonate, and the still strongly acid liquor was thoroughly extracted with ether. The ethereal solution was now thoroughly extracted with dilute caustic soda, and on evaporating the ether nearly pure coprostanone was obtained in a yield in all experiments of about 70 per cent.

The caustic soda extracts on acidification with hydrochloric acid gave a white crystalline precipitate of an acid mixed with an oily substance. The oil was got rid of by washing with petroleum ether, in which the crystals were insoluble. During the extraction of the ethereal solution of the oxidation product with caustic soda a small quantity of an insoluble basic chromium salt also separated, and this on treatment with hot hydrochloric acid yielded a further quantity of the crystalline acid. This acid is soluble in ether, but only difficultly soluble in benzene and in chloroform. It is sparingly soluble in hot acetone and somewhat more readily in boiling ethyl acetate or alcohol. It can be readily purified by recrystallisation from a mixture of ether and acetone or from ethyl acetate. It usually separates as a fine powder, and under the microscope appears in the form of well-defined square rods. It melts at 247° to a clear liquid, without any decomposition. For analysis the substance was dried in vacuo. It proved rather difficult to burn.

(1) 0·2054 g.; 0·5604 CO_2; 0·1934 H_2O.
(2) 0·2433 g.; 0·6628 CO_2; 0·2358 H_2O.

Found (1) C=74·41; H=10·46; (2) 74·30; 10·76. Calc. for $C_{27}H_{44}O_4$, C=74·94; H=10·26; $C_{27}H_{46}O_4$, C=74·59; H=10·67.

In other combustions in which the carbon came low, the hydrogen was 10·38, 10·23 and 10·63.

Sodium salt. On titration with caustic soda in alcoholic solution 0·6304 g. required 14·43 cc. N/5 NaOH. With another specimen 0·3134 g. required 7·24 cc. N/5 NaOH. Mol. wt. found (1) 436·8, (2) 432·8. Calc. for $C_{27}H_{46}O_4$, 434.

Ammonium salt. The acid dissolved in strong ammonia giving a soapy solution. The bulk of the free ammonia was boiled off, and on cooling the liquid set to a perfectly clear stiff jelly. The jelly dried up in vacuo, with gradual evolution of ammonia, to a white amorphous mass, which dissolved in hot water giving a slightly opalescent soapy solution. For analysis the substance, dried finally at 100°, was decomposed by excess of standard acid, filtered and the acid titrated back with soda. 0·3157 g. was decomposed with 25 cc. N/5 sulphuric acid and the excess of acid required 42·5 cc. soda. 25 cc. N/5 acid = 49·2 soda; percentage of N = 3·19. Calc. for $C_{27}H_{49}O_4N$ = 3·1 %.

Silver salt. This was prepared by precipitating a neutral solution of the sodium salt with silver nitrate. The white precipitate was well washed with water, in which it was quite insoluble, and dried at 100°.

(1) 0·298 gave on ignition 0·0998 g. of silver.
(2) 0·1467 ,, ,, 0·0489 ,, ,,

Mol. wt. of acid found (1) 430·6. (2) 433·8. Calc. for $C_{27}H_{46}O_4$, 434.

From the analysis of these salts and the combustions of the acid itself, the acid appears to be a dibasic acid of the formula $C_{27}H_{46}O_4$.

The oil formed along with this acid and dissolved away by petroleum ether as described above, would not crystallise on long standing. It was an acid and formed an insoluble barium salt on precipitating the solution in ammonia with barium chloride. This substance has not yet been further investigated.

Oxidation of coprostanone by chromic acid. In order to ascertain whether the acid $C_{27}H_{46}O_4$ was a bye-product in the preparation of coprostanone, or formed by the further oxidation of the coprostanone by the excess of chromic acid, one gram of coprostanone was dissolved in glacial acetic acid and heated to 70° with 0·35 gram of chromic anhydride dissolved in dilute acetic acid until all the red colour had gone. It was then heated to boiling, cooled and poured into water. The turbid liquid was extracted with ether, and on evaporating the ether a green oil containing chromium was obtained. This was freed from chromium by warming with hydrochloric acid on the water bath. The liquid was then extracted with ether, the ethereal solution evaporated and the brown oil left taken up with petroleum ether. The petroleum was allowed to evaporate spontaneously and left a brown oil, which on long standing in a desiccator deposited some crystalline matter. The oil was dissolved away by petroleum ether, and the crystalline matter recrystallised from hot ethyl acetate. It melted at 247° and was identical with the acid described above. The yield was however small. The oil dissolved by the petroleum was acid in character and formed an insoluble barium salt.

Oxidation of coprostanone by ammonium persulphate. As the yield of acid $C_{27}H_{46}O_4$ was small by both methods of preparation, we thought it desirable to try other oxidising agents and ultimately selected ammonium persulphate. This reagent is known to act on cyclic ketones [Baeyer and Villiger, 1899] breaking the ring and changing them to hydroxy-acids or their lactones; and we hoped in this way to break the ring of the coprostanone at the CO group and obtain a hydroxy-acid which might on further oxidation give the dibasic acid $C_{27}H_{46}O_4$. 10 g. coprostanone were dissolved in 375 cc. of glacial acetic acid, and to the solution were added 10 g. ammonium persulphate, dissolved in the least possible amount of water and diluted with an equal volume of glacial acetic acid. The mixture was then heated on a water bath and another 10 g. of powdered ammonium persulphate gradually added. The heating was continued for four hours, during which gas was slowly evolved. This gas was mainly oxygen from the persulphate, but contained some carbon dioxide. After standing several days the liquid was

diluted with an equal volume of water and thoroughly extracted with ether. The ethereal solution was repeatedly shaken with water to get rid of the bulk of the dissolved acetic acid, and then extracted with 10 % caustic soda solution several times, until a sample of the alkaline extract no longer gave a precipitate on acidification. The soda solution on shaking with the ethereal extract turned dark brownish red in colour. The various caustic soda extracts were mixed and on standing overnight bulky gelatinous clots of a colourless sodium salt separated out (A). These were somewhat difficult to filter, but it was found possible to syphon off the greater portion of the mother liquor. The mother liquors and filtrates, which were now pale yellow, on acidification with hydrochloric acid gave an amorphous precipitate of an acid substance (B). The ethereal solution, freed from acid matter, gave on evaporation a small quantity of a neutral substance (C).

Examination of (*A*). The clots were readily soluble in hot water giving a clear soapy solution, from which they separated on cooling, but more readily if the solution was made strongly alkaline. On acidifying the aqueous solution with hydrochloric acid a crystalline precipitate was thrown down which, in the preparation described, amounted to about 65 % of the weight of the coprostanone taken. In different preparations, however, the relative amounts of (A), (B) and (C) varied considerably with the conditions of reaction.

A small quantity of the clotted matter was filtered on the pump, washed with cold water to get rid of excess of alkali and further purified by means of alcohol. It was obtained as a white powder perfectly soluble in hot water. For analysis it was dissolved in water and precipitated by a measured quantity of standard acid. The precipitate was filtered, well washed and the filtrates titrated with alkali.

0·2488 g. was found to contain sodium equivalent to 6·1 cc. N/10 caustic soda; percentage sodium = 5·64. Calc. for $C_{27}H_{47}O_3Na = 5·21$.

The crystalline precipitate obtained on acidifying the aqueous solution of this sodium salt can be separated into two substances by fractional crystallisation from ethyl acetate or better from alcohol. The less soluble substance, present in small quantity, crystallised in thin plates and after several crystallisations melted at 183–184°. The more soluble substance, constituting the bulk of the material, crystallised in minute needles and melted at 157–158°.

Substance melting at 157–158°. This body was easily soluble in ethyl acetate, acetone and benzene, but somewhat less so in alcohol. It was sparingly soluble in petroleum ether, but more soluble in the fraction of

petroleum boiling at 156–195°. On combustion the following results were obtained :—

(1) 0·1549 ; 0·4568 CO_2 ; 0·1595 H_2O.
(2) 0·1571 ; 0·4625 ,, ; 0·1617 ,,
(3) 0·1640 ; 0·4834 ,, ; 0·1680 ,,
(4) 0·2557 ; 0·7503 ,, ; 0·2627 ,,

	I	II	III	IV	Calc. for $C_{27}H_{46}O_2$
C	80·42	80·29	80·40	80·02	C 80·52
H	11·44	11·43	11·38	11·41	H 11·50

Combustion does not however throw much light on the number of carbon atoms in the molecule, as decrease of the molecular weight by CH_2 makes very little difference in the percentage composition thus—

			C	H
$C_{27}H_{46}O_2$	...	...	80·52	11·50
$C_{26}H_{44}O_2$	...	...	80·34	11·42
$C_{25}H_{42}O_2$	...	...	80·14	11·31
$C_{24}H_{40}O_2$	...	...	79·93	11·19

The molecular weight was therefore determined by depression of the freezing point of benzene :—

(1) 0·2975 g. in 20 cc. benzene of sp. gr. 0·883, depression of freezing point 3·115° to 2·915°.
(2) 0·5993 g. in 15 cc. benzene of sp. gr. 0·883, depression of freezing point 3·115° to 2·565°.

Molecular weight found I 412·7. Calc. for $C_{27}H_{46}O_2$, 402·37.
II 403·1.

The substance did not go into solution on boiling for a short time with an aqueous solution of caustic soda, nor on shaking a solution in ether with either 10, 20 or 30 % caustic soda, though as originally obtained in the acetic acid solution it did so quite readily. It apparently contained no OH group, as it was quite unaffected by prolonged boiling with either acetic anhydride or acetyl chloride, and on pouring into water and extracting with ether it was recovered unchanged in both cases. It was probably a very stable lactone of a hydroxy-acid of the formula $C_{27}H_{48}O_3$.

Examination of (*C*). The neutral substance left in the ether after extraction with caustic soda, can be readily purified by recrystallisation from alcohol, or ethyl acetate, or petroleum ether. It melted at 183–184° and was identical with the substance of the same melting point obtained along with the lactone melting at 157–158°. This was proved by a mixed melting point. It crystallised in long thin plates, or glistening leaves, which under the microscope appeared to be rectangular plates, some square, some long. It proved difficult to burn and on combustion gave the following results :—

(1) 0·1197; 0·3514 CO_2; 0·1235 H_2O.
(2) 0·1720; 0·5052 ,, ; 0·1672 ,,
(3) 0·1455; 0·4264 ,, ; —
(4) 0·1580; 0·4615 ,, ; 0·1594 ,,

	I	II	III	IV
C	80·06	80·11	79·92	79·66
H	11·46	10·80	—	11·21

0·8365 g. in 15 cc. benzene of sp. gr. 0·883, depression of freezing point 0·76°. Molecular weight found, 407; calc. for $C_{27}H_{46}O_2$, 402·37. The substance was not affected by shaking in ethereal solution with either 10, 20 or 30 % caustic soda solution.

The analyses threw no light on the relationship of the substance to the lactone melting at 157–158°. The molecular weight determination perhaps suggests that it is a stable lactone isomeric with the 157–158° body. It may however be a lactone of lower molecular weight produced by further oxidation, or possibly some derivative of a ketonic or diketonic nature. To test this 0·95 g. of the lactone (M.P. 156°) was dissolved in glacial acetic acid and 5·7 cc. of 10 % chromic acid solution added. The mixture was kept at 70° for half an hour, and then the temperature was raised to the boiling point for a few minutes. The liquid was then poured into cold water and the precipitated matter filtered off. The filtrate was extracted with ether and a further quantity of solid obtained, which was added to the precipitate. This precipitate was crystallised from alcohol from which it separated in needles and plates, melting at 169–171°. This weighed 0·38 g. or 40 % of the weight of the original substance. After several recrystallisations it melted at 183–184°. This was shown by a mixed melting point determination to be identical with the substance melting at 183–184° obtained from (C). The lactone (M.P. 157–158°) appears therefore to yield this neutral substance melting at 183–184° on oxidation. The latter body itself on similar treatment did not appear to be further oxidised, and was recovered unchanged. It did not appear to have ketonic properties for the following reasons: (1) sodium amalgam had no effect on the solution of the substance in moist ether in presence of sodium bicarbonate; (2) in attempts to prepare a semicarbazone in the usual manner, the substance was recovered unchanged; (3) on heating 1 mol. of the substance in alcoholic solution with 6 mols. of hydroxylamine hydrochloride and the equivalent amount of dry sodium acetate for 20 hours, the substance was recovered quantitatively and unchanged. On repeating experiment (3) however using caustic soda instead of sodium acetate, needle shaped crystals were obtained on evaporating the alcohol. These melted at 268–274°. This was not however an oxime as it contained only a trace of

nitrogen—under 1 %. The same or a very similar substance, melting at 264–286°, was also obtained on substituting phenylhydrazine hydrochloride for the hydroxylamine in the above experiment. This contained about 0·9 % of nitrogen. We had not sufficient material for the further investigation of these substances. It would seem probable however from these results that the substance of M.P. 183–184° is a stable lactone either isomeric with or containing less carbon than the lactone of M.P 157–158°.

Examination of substance (*B*). The acid precipitated on acidifying the alkaline solution was a light brown amorphous resin-like substance, readily soluble in alkalis giving a soapy solution. It was easily soluble in the common organic solvents, but would not crystallise. From petroleum ether it dried up to a brittle resin. It was dissolved in ammonia and reprecipitated by hydrochloric acid several times, and well washed. After drying it melted at 88° to a stiff paste and decomposed at 114°. The yield was only small.

Barium salts. The resinous acid was dissolved in ammonia, the excess of alkali exactly neutralised by hydrochloric acid and the solution precipitated with baryta water. The precipitate, after being well washed with water, dried to a light brown powder. This was readily soluble in chloroform, and when the solvent was allowed to evaporate spontaneously separated on the sides of the beaker in light brown brittle flakes. On analysis—

(1) 0·1367; 0·0327 $BaSO_4$.
(2) 0·2173; 0·0494 ,,
(3) 0·3541; 0·8468 CO_2; 0·3047 H_2O.

	Ba	C	H
I	14·11	—	—
II	13·50	—	—
III	—	65·22	9·56
Calc. for $C_{27}H_{45}O_4Ba_{1/2}$	13·68	64·53	9·03
Calc. for $C_{54}H_{93}O_7Ba$	13·83	65·25	9·64

The filtrate obtained after precipitating with baryta in the preparation of the above salt was acidified with hydrochloric acid and the small amount of amorphous resin precipitated was filtered and well washed. It was then dissolved in hot baryta water and the excess of baryta precipitated by carbon dioxide. The milky liquid was well boiled and the barium carbonate filtered off. The filtrate was evaporated to small bulk and left in a desiccator. Crystals gradually separated, which under the microscope appeared to be small plates. There was only sufficient for a barium estimation after drying at 100°.

0·1424; 0·0590 $BaSO_4$; Ba found = 24·39; Calc. for $C_{27}H_{44}O_4Ba$ = 24·12; $C_{54}H_{93}O_7Ba_2$ = 24·35.

We hope shortly to be in a position to give a further account of the reactions of these various substances.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work.

REFERENCES.

Baeyer and Villiger (1899), *Ber.* **32**, 3625.
Dorée and Gardner (1908), *J. Chem. Soc.* **93**, 1629.
Dorée (1909), *J. Chem. Soc.* **95**, 653.

LVI. A NOTE ON A MODIFICATION OF TEICHMANN'S TEST FOR BLOOD.

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(*Received November 4th, 1913.*)

For the identification of blood as such, several tests are in common use. But tropical conditions, such as are found in Ceylon, in many cases render some of these tests of little value. Hence I do not hesitate to put forward this modification of an old test, as I have found it to be very satisfactory.

The routine methods which may be adopted in cases of suspected blood stains are as follows:

I. *The benzidine test.* Benzidine is freshly dissolved in a small quantity of glacial acetic acid and hydrogen peroxide is added. This will give an *immediate* blue colour if it comes in contact with any blood. As, however, the immediate blue colour is produced by some other substances, the test merely serves to weed out a large number of stains which superficially resemble blood stains.

II. *The spectrum test.* In ordinary routine work it has been found that in suitable cases good results are obtained by soaking out the stain with ammonium hydrate and then adding ammonium sulphide to obtain the haemochromogen spectrum. But with very small stains there is often insufficient material to get the spectrum without special microscopical apparatus. In addition, when the stains have been long exposed to the tropical sun, they are apparently rendered so insoluble that the pigment is broken up before a solution can be obtained for the spectroscope. Further, in cases where the stain has been rusted on to iron and is not in large quantity, it has been found to be practically impossible to obtain a solution which will yield a spectrum.

III. *The corpuscle test.* This has been found to be of very doubtful value and very often impracticable in the case of old stains.

IV. *Teichmann's haemin crystal test.* This at first sight appears to provide a very convenient and reliable method of making a complete

identification of blood as such. In routine practice in the tropics, however, I understand that it has been largely discontinued, in consequence of the hitherto insuperable difficulties, which arise under certain conditions. As in the case of the spectrum test, old stains which have been exposed for a long time to air and sun in the tropics, and stains which are rusted on to iron, usually give negative results, even in cases where it is known that the stains were caused by blood.

Therefore I considered it of interest to examine the literature on the subject, and to evolve if possible some modifications which would give better results. The routine method in vogue had been to use glacial acetic acid with a trace of sodium chloride. Following the recommendations of various writers, I used, in place of sodium chloride, at first potassium iodide, and later sodium iodide with the glacial acetic acid. Good results were obtained with fairly fresh blood, but the results in the above mentioned obstinate cases were still unsatisfactory. So an attempt was made to substitute some other acid for the acetic acid.

Sulphuric, formic, valeric, butyric, and several other acids and combinations of acids and salts were tried without much success. Eventually strong lactic acid (sp. gr. 1·21 Kahlbaum) was tried with sodium iodide. This acid is mentioned in the literature as having been tried by Teichmann himself.

At first a very small fragment of the stain was placed in a drop of a solution of sodium iodide (approx. 5 %) in water, on a glass slide. This was carefully dried on an asbestos mat over a Bunsen flame. The cover glass was then placed over it and the lactic acid allowed to run under the cover glass, so as to cover the stain. The slide was then warmed on the asbestos mat and examined for crystals. Excellent results were obtained.

Later, solid sodium iodide, freshly prepared, was dissolved in the lactic acid to about 1 %. This solution becomes brown on keeping, but the change does not appear to impair its action, at any rate for some months. The fragment of stain is covered with the solution, and the cover glass is placed in position, and the whole slowly warmed as above to such a temperature that the solution is just about to boil under the cover glass. In most cases five minutes treatment is sufficient to produce crystals. If they have not then appeared, the heating must be continued; more solution must be allowed to run under the cover glass if necessary, but this is not often the case, as the lactic acid does not evaporate as rapidly as acetic acid. The resulting crystals differ very much in size in different cases, being usually rather small, but dark and perfectly characteristic.

By this method I have not failed to obtain crystals, in any case which

I have tried up to the present. Stains on filter paper which had been kept for three years exposed to the air, and were then exposed to direct sunlight for some hours, gave excellent results. An old stain, rusted on to a large iron knife, also gave perfectly clear results. These two failed to give any results with the other tests, except with benzidine.

One great advantage of the method is that only the very smallest fragment of stain is necessary. In fact it is essential for the production of good results that only a small amount of blood be taken. But it has also been found rather essential that the blood should not be very dilute. The smallest scraping from a solid article, or a shred of stained material about a millimetre long, is quite sufficient.

In using fresh blood it is advisable to dry the blood first.

Summary.

The benzidine test for blood stains is merely a preliminary test as it is not characteristic.

The spectrum and corpuscle tests fail in many cases, as does the ordinary method of using Teichmann's test. The use of a mixture of sodium iodide and lactic acid is recommended for the latter, in place of sodium chloride and acetic acid.

LVII. ON THE BEHAVIOUR OF AMYLASE IN THE PRESENCE OF A SPECIFIC PRECIPITATE.

By AGNES ELLEN PORTER,

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(*Received Nov. 10th, 1913.*)

The complement-binding phenomenon which takes place in the presence of antigen and antibody has become of great practical importance in the recognition of disease. The difficulties of the test are great, owing to the fact that five different, chemically impure, and little known substances must be employed: antigen, antiserum, complement, amboceptor, and red corpuscles. The last three components are very troublesome to obtain, and it is not surprising that many efforts should be made to minimise this trouble, and to lessen the difficulties which are necessarily dependent on such a complicated process. The most attractive simplification would be one in which all these three components of the haemolytic system, viz. complement, amboceptor, and red corpuscles, are entirely done away with. This would be possible if some substance could be found which would become fixed, like complement, by the antigen-antibody mixture, but whose absence could be tested for more easily.

Like complement, animal ferments seem to be very rapidly absorbed by coagulated colloids and even by organic precipitates of different kinds. The ferment shows some selective action in this tendency. Pepsin absorbed into egg-white [Dauwe, 1905, p. 426] or pepsin, ptyalin, etc. into collodion [Porter, 1910, p. 382] can be recovered in some degree by a solution of the particular substance which they digest. As ferments can only be recognised through their effects, this possibility of partial recovery presents the chief objection to the use of ferments as substitutes for complement.

Most ferments are powerfully influenced by the presence of serum. Proteolytic ferments are inhibited by serum, probably because their attraction to the serum proteins, even in a dissolved condition, is already so great. On the other hand other ferments, such as starch-splitting ferments and lipases, are markedly accelerated by the presence of serum.

Hailer [1908, p. 280] first made the attempt of absorbing a ferment by means of a specific precipitate. He chose rennet, with sheep's serum as antigen, and the serum of a rabbit immunised against sheep's protein as antibody. This choice was perhaps unfortunate, as not only rennet but also antirennet have been described in serum [Fuld and Spiro, 1900, p. 141]. Also, as I have pointed out, rennet suffers very little from dilution, when the dilution has been freshly made. Rennet has indeed acted perfectly in my hands [1911, p. 394] at a dilution of 1/340,000. It can therefore be almost entirely absorbed without any evidence of the fact. On this account I thought it advisable to attempt the absorption with another ferment. Pepsin and trypsin are inactivated by serum; I therefore chose amylase. Szumowski [1898, p. 162] has proved that amylase is absorbed by fibrin. It is much less easily absorbed by serum proteins in their natural state, as serum is itself amylolytic. The advantages of the ferment were therefore as follows:

(1) It is present in serum, so that, were the absorption successful, no further addition of ferment to the antiserum would be required.

(2) It is apparently not absorbed by serum proteins in their normal state, i.e. before the union of antigen and antibody.

(3) It loses rapidly on dilution, so that even a small loss could be measured.

Method.

Ferment, guinea-pig-serum, saliva, and taka-diastase.

Antibody, serum of rabbits immunised against egg-white or against horse-serum.

Antigen, egg-white or horse-serum.

The ferment, in as small bulk as possible, i.e. 0·05 cc. saliva, or 0·15 cc. diluted taka-diastase, was added to 0·15 cc. immune or normal serum, and 0·15 cc. of the various dilutions of egg-white or horse-serum, the mixture being left at room temperature overnight. Next morning 3 cc. of a 1 % soluble starch solution were added, and the tubes were placed at 37° for 5 to 20 minutes, or in the case of guinea-pig-serum for a half to one and a half hours. The mixtures were then tested for sugar by means of Fehling's solution, and for dextrins by means of iodine. On account of the protein present, use was made of the dialysed iron method, which had the double advantage of removing both protein and ferment. When the mixtures were taken from the incubator, they were made up to a bulk of 5 or 6 cc. with physiological saline solution, and 0·5 cc. saturated salt solution, and 0·5 cc.

B.P. Liquor Ferri Dialysatus were added to each and the resulting precipitate immediately filtered off. A measured amount of the perfectly clear and inactive filtrate was then tested quantitatively for sugar. The colour given by the iodine test in the filtrate was clear and permanent.

The difference between the effect of immune and normal serum on amylase in the presence of antigen was very slight, but on account of the accuracy of the method and the number of times the experiment has been repeated, it may be taken as the expression of a genuine though very partial absorption.

Absorption experiment.

A. *Immune serum.* Rabbit immunised against egg-white.
(To each tube were added 0·05 cc. saliva, 0·15 cc. anti-egg-serum, 0·15 cc. diluted egg-white, method as above.)

Dilution of egg-white	Time at 37° with starch	Cc. of Fehling sol.	Iodine solution 0·5 cc.
1/10	10 minutes	0·2	Dark mauve.
1/50	,,	0·225	,,
1/100	,,	0·225	,,
1/250	,,	0·25	,,
1/500	,,	0·25	Light mauve.
NaCl	,,	0·3	Pale pink.
1/10	20 minutes	0·25	Dark mauve.
1/50	,,	0·25	Medium mauve.
1/100	,,	0·25	,, ,,
1/250	,,	0·275	Medium light mauve.
1/500	,,	0·3	Light mauve.
NaCl	,,	0·35	Very pale.

B. *Normal rabbit-serum.*
(To each tube were added 0·05 cc. saliva, 0·15 cc. normal serum, 0·15 cc. diluted egg-white, method as above.)

1/10 to 1/500	10 minutes	0·3	All pale.
NaCl	,,	0·3	Pale.
1/10 to 1/500	20 minutes	0·375	,,
NaCl	,,	0·375	,,

When serum is used as antigen a curious phenomenon may be noticed. In this case all three ingredients, antigen, antibody, and saliva, contain amylase from three separate species, and this gives rise to an acceleration which is decidedly beyond the sum of the three separate activities. Although the antiserum was heated at 56° to reduce its amylolytic power, it was still able to exert an accelerating influence. In the following experiment, where the antigen is horse-serum, acceleration is to be seen and must be allowed for. In spite of it, absorption can be observed if the "immune" and "normal" columns are compared.

A. *Rabbit-serum*, immune to horse-serum.

(To each tube were added 0·05 cc. saliva, 0·15 cc. anti-horse-serum, 0·15 cc. diluted horse-serum, method as above.)

Dilution of horse-serum	Time at 37° with starch	Ccs of Fehling sol.	Colouration with iodine solution
1/10	10 minutes	0·5	Red mauve.
1/50	,,	0·45	,,
1/100	,,	0·4	,,
1/250	,,	0·4	Same, but deeper.
1/500	,,	0·35	Mauve.
NaCl	,,	0·35	,,
1/10	20 minutes	0·5	Pale pink.
1/50	,,	0·5	,,
1/100	,,	0·45	,,
1/250	,,	0·4	Mauve.
1/500	,,	0·35	,,
NaCl	,,	0·35	,,

B. *Normal rabbit-serum.*

(To each tube were added 0·05 cc. saliva, 0·15 cc. normal rabbit-serum, 0·15 cc. diluted horse-serum, method as above.)

1/10	10 minutes	0·55	Colourless.
1/50	,,	0·55	,,
1/100	,,	0·5	,,
1/250	,,	0·4	Pale.
1/500	,,	0·35	Mauve.
NaCl	,,	0·35	,,
1/10	20 minutes	0·6	Colourless.
1/50	,,	0·6	,,
1/100	,,	0·55	,,
1/250	,,	0·45	,,
1/500	,,	0·4	Pale.
NaCl	,,	0·35	Mauve.

Taka-diastase displayed no tendency whatsoever to become absorbed by a specific precipitate. This is interesting, as I have noticed before [1910, p. 386] that while ptyalin was inactivated by collodion membranes in a day, taka-diastase was hardly affected in a month's time.

Behaviour of Serum Amylase on Precipitation with Carbon Dioxide.

Fresh guinea-pig-serum, diluted to 1/10 in water, was saturated with carbon dioxide, and the globulins separated and redissolved in saline solution, after centrifuging. The upper fluid taken from the centrifuge had its isotonicity restored by sodium chloride, and both portions were tested for amylase. The ferment was found to be almost unaffected by the precipitation, practically the whole ferment remaining free in the upper fluid.

Time 1½ hours	Cc. of Fehling sol.	Colouration with iodine
1 cc. serum 1/10 + 1 cc. NaCl 0·85 %	0·5	Brown.
1 cc. precipitate (1/10) + 1 cc. NaCl 0·85 %	0·05	Dull, dark.
1 cc. upper fluid + 1 cc. NaCl 0·85 %	0·4	Brown.
1 cc. serum 1/10 + 1 cc. precipitate	0·475	,,
1 cc. serum 1/10 + 1 cc. upper fluid	0·65	Colourless.
1 cc. precipitate + 1 cc. upper fluid	0·425	Brown.

Although these experiments have been attended with little success, I am venturing to record them as of some small theoretic interest, and partly to recommend a good method for testing amylolytic action in the presence of serum.

REFERENCES.

Dauwe (1905), *Beiträge*, **6**, 426.
Fuld and Spiro (1900), *Zeitsch. Physiol.* **31**, 132.
Hailer (1908), *Arbeit. kaiserl. Gesund.-Amt.* **29**, 277.
Porter (1910), *Quart. J. exp. Physiol.* **3**, 375.
—— (1911), *J. Physiol.* **42**, 389.
Szumowski (1898), *Arch. Physiol.* 5e série, 160.

LVIII. THE GALACTOSIDES OF THE BRAIN. I.

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(*Received Nov. 10th, 1913.*)

Nearly forty years ago Thudichum [1874] recognised the existence in the brain of substances analogous in their constitution to the vegetable glucosides. He distinguished two principal representatives of this class which he called phrenosin and kerasin. Phrenosin being relatively easy to prepare in sufficient amount for further study, Thudichum succeeded in elucidating its constitution by an investigation of its hydrolytic cleavage products. The carbohydrate obtained on hydrolysis of phrenosin was originally called "cerebrose" by its discoverer, but was identified later with galactose [Thierfelder, 1890; Brown and Morris, 1890]. The group name "cerebroside," in analogy to "glucoside," was introduced by Thudichum on the assumption that cerebrose was the typical carbohydrate of the brain, but later on he himself adopted the name cerebro-galactoside. In view of the fact that the occurrence of these substances is not limited to the brain[1], the use of the general name "galactoside" seems to be preferable to the special term "cerebroside."

The work of subsequent investigators was limited, until quite recently, to the preparation by modified methods of the substances first obtained by Thudichum, without contributing to the knowledge of their cleavage products. Unfortunately a great confusion in the nomenclature of these substances has resulted from the failure of earlier workers to co-ordinate their results with each other and with those of Thudichum. It seems that too much importance was attached to minor differences in the figures of elementary analysis and to variations in the melting points of these substances of high molecular weight; it was mainly on this basis that new names like pseudo-cerebrin, cerebrin, homocerebrin, cerebron were introduced for substances, which from the method of their preparation were nothing else than Thudichum's original phrenosin and kerasin, or mixtures of the two.

[1] Similar substances have since been obtained from pus (Kossel and Freitag), from blood (Bang and Forssmann), from adrenals (Rosenheim and Tebb), and also from certain mushrooms (Zellner), etc.

In the course of a prolonged investigation of these substances, carried out during the last six years, the author has been able to confirm Thudichum's main observations and has worked out a new method for the preparation of the galactosides from brain. The complete separation of the galactoside mixture into its two principal constituents, phrenosin and kerasin, was made possible for the first time by the help of a new physical test, depending on the behaviour of these substances under the polarising microscope, which will be described later.

The investigation of the optical activity of the two substances brought out an interesting difference between them. Whilst phrenosin, in confirmation of the previous observations of Thierfelder and Kitagawa [1906], proved to be dextro-rotatory, it was found that kerasin, when completely separated from all dextro-rotatory phrenosin, possessed a laevo-rotation. Levene and Jacobs [1912, 2] and subsequently Thierfelder [1913] have since described as inactive a "kerasin fraction" prepared by different methods and evidently not completely freed from phrenosin.

The two products were further subjected to hydrolysis and all the cleavage products were identified. As far as phrenosin is concerned, the results agreed with Thudichum's observations which had already been confirmed in the main outlines by Thierfelder's work [1904, 1905].

The complete hydrolysis of kerasin had hitherto not been carried out and its relationship to phrenosin had remained unexplained. In its elementary composition it agrees closely with phrenosin and yet it differs widely from it in appearance, solubility and other physical constants. Thudichum suggested that the essential difference between it and phrenosin might be due to a difference in the fatty acid radicle. Neither the fatty acid nor the carbohydrate nor the base had been isolated or identified by Thudichum. Levene and Jacobs [1912, 2] recently advanced the hypothesis that the two substances represented optical isomers.

My own results show that Thudichum's suggestion was correct and that kerasin contains a different fatty acid from phrenosin. On hydrolysis of kerasin, purified as far as possible, I obtained as the only fatty acid one of the composition $C_{24}H_{48}O_2$, which I was able to identify with lignoceric acid [1913]. The carbohydrate was found identical with *d*-galactose and the base with sphingosine, as was to be expected from Thudichum's preliminary work.

Phrenosin, on the other hand, gives rise to the optically active hydroxy-acid $C_{25}H_{50}O_3$, as well as to *d*-galactose and sphingosine[1].

[1] Thierfelder [1904] was the first to show that the fatty acid obtained by Thudichum from phrenosin represented a hydroxy-acid. Levene and Jacobs [1912, 1] satisfactorily explained the

In connection with this work the question was investigated in which form the galactosides occur in the brain, and it is proposed to deal with this fundamental question first, leaving the description of the methods used for the separation of phrenosin and kerasin, as well as for their purification and hydrolysis, for a subsequent communication.

I. The galactosides exist in the brain in the free state.

Previous investigations leave the question undecided whether the galactosides occur preformed in the brain or whether they are split off from more complex substances during the process of preparation. Whilst some recent authors incline to the view that part at least exist preformed, others are of the opinion that, as only small quantities are obtainable directly from the brain without drastic methods, they owe their origin to the partial decomposition of a preformed complex substance. No experimental proof for either view has been brought forward.

It is evident that this question cannot be decided by the use of those methods hitherto employed for the preparation of the galactosides, in which either baryta is used for the removal of the phosphatides, or in which solvents like alcohol are used at their boiling temperature. In both cases a decomposition of a preformed combination is possible. It seemed feasible, however, to obtain conclusive evidence on this point by the use of an inert solvent which dissolves galactosides in the cold, and in which phosphatides and sulphatides are insoluble. Such a solvent was found by Rosenheim and Tebb [1910] in pyridine.

In applying the pyridine method directly to brain, it is preferable previously to remove water and cholesterol by means of cold acetone [Rosenheim, 1906], and the unsaturated phosphatides, lecithin and kephalin, by means of ether or petroleum ether. As the same method was used subsequently for the isolation of the galactosides, the procedure adopted may be described here in detail.

differences in melting point of Thudichum's and Thierfelder's acid by showing that the acid existed in two isomeric modifications, of which the optically inactive one melts at 82–85° (Thudichum's "neurostearic" acid), whilst the dextro-rotatory form melts at 106–108° (Thierfelder's "cerebronic" acid). From a "kerasin fraction" Thierfelder [1913] has recently obtained an acid of the composition $C_{24}H_{48}O_2$; Levene [1913] has since identified a similar acid as lignoceric acid, which he isolated on hydrolysis of a "cerebrin fraction." Both Thierfelder's and Levene's fractions were optically inactive and contained therefore probably dextro-rotatory phrenosin admixed with the laevo-rotatory kerasin. Thierfelder, indeed, obtained 6 % of cerebronic acid, the typical acid of phrenosin, on hydrolysis of his "kerasin fraction." The carbohydrate had not been identified by either of these workers.

10 kg. of finely minced ox brain were suspended in 10 litres of acetone and allowed to stand with frequent stirring for 24 hours at room temperature. The watery acetone extract was decanted and the brain pulp strained through several layers of fine muslin. At least six subsequent extractions with sufficient acetone to cover the tissue were made, until the last extract on evaporation yielded only an inappreciable amount of cholesterol. The total quantity of crude cholesterol obtained from these extracts varies between 240–260 g., i.e. about 2·5 % of the fresh brain.

The tissue was next spread in a thin layer on large glass plates, gently warmed from below, and freed from acetone by means of an air current (electric fan).

The dry, somewhat waxy powder was now subjected to extraction with cold petroleum ether. Five to six extractions were usually found sufficient to remove the unsaturated phosphatides, which may be obtained from the extracts by the usual methods. After the removal of the petroleum ether by an air current in the way described above, the tissue was passed through an Excelsior mill and was thus obtained as a fine cream-coloured powder, ready for the pyridine treatment. 10 kg. of fresh brain yield on the average 1300 g. (from 1200–1400 g.) of this powder, which seems to keep indefinitely in this condition.

For the preparation of the galactosides it was found convenient to work up 500 g. of this powder at a time. This quantity was covered with 1500 cc. of pyridine (B.P. 115°) and after having been warmed to 45° by being kept for about twenty minutes in a water-bath at 50°, it was rapidly cooled to room temperature. Although the galactosides are readily soluble in cold pyridine, the initial warming is necessary in order to allow the solvent to penetrate the tissue. Filtration proceeds easily by means of a large Buchner funnel. From their pyridine solution the galactosides were obtained by pouring it into 3–4 volumes of acetone[1]. A bulky white precipitate is formed, from which the supernatant fluid can be easily decanted off after standing some time. The mother liquor deposits still further on cooling on ice. In the earlier experiments this deposit was filtered off separately, but as the amount was found to be very small, the pyridine-acetone mixture was cooled down directly to 0° in the later experiments.

The filtration and washing of the mixed galactosides under pressure proceeds very slowly and it is therefore advisable to filter through a plain filter. After thorough washing with acetone, the precipitate is suspended in acetone and only then filtered under pressure. After being dried in vacuo,

[1] The pyridine solution may, of course, be previously concentrated by distillation in vacuo.

the powder is extracted with ether in a Soxhlet, in order to remove the last traces of ether-soluble phosphatides.

The crude galactosides are thus obtained as a slightly yellowish powder, the average yield amounting after two extractions to 205 g. from 10 kg. of moist brain, i.e. 2 per cent.

It is of interest to compare this experimental yield with the theoretical. Unfortunately there is at present a lack of a reliable quantitative method for the estimation of galactosides and the data available in the literature as to the percentage of galactosides in normal brain are correspondingly scanty. Probably the method worked out recently by Lorrain Smith and Mair [1913], in which the galactosides are weighed as such, gives truer results than the older methods in which the reducing power of hydrolysed brain extracts served as a basis of calculation. Lorrain Smith and Mair state their results in percentages of the chloroform extract of dried brain. Taking the mean water content of brain as 78 %, I have calculated from their results the percentages of galactosides as 7·3 % in dry and 1·6 % in fresh normal human brain. This result agrees well with the experimental yield of 9·8 % in dry and 2 % in fresh brain, as stated above, if we consider that these figure refer to the crude product.

After having been recrystallised twice from 15 volumes of an alcohol-chloroform mixture (1 : 2) the substance was obtained as a white powder, which contained 1·68 % of nitrogen and only a very small percentage of phosphorus (0·08 %).

0·3000 g.; 2·36 cc. N/10 KOH by Neumann's method.
0·4893 g.; 5·86 cc. N/10 KOH by Kjeldahl's method.

The product represents, as will be shown in a subsequent communication, a mixture of at least two substances. For the purpose of the present investigation it was considered sufficient to show that it consists essentially of galactosides, and the substance was therefore subjected to hydrolysis without any further purification.

Method of estimation of the products of hydrolysis.

Hydrolysis was carried out in methyl alcohol solution with sulphuric acid, and the cleavage products estimated in a way similar to that described by Thierfelder [1905] for "cerebron." Galactose was estimated polarimetrically and the fatty acids (as esters) and bases (as sulphates) were collected as carefully as possible and weighed.

1 g. of the substance was dissolved in 50 cc. methyl alcohol containing

5 cc. concentrated sulphuric acid and boiled under a reflux condenser on a water bath for six hours. After standing overnight, white glittering scales of the esters (and free fatty acids) had crystallised out. The flask was kept in the ice-chest for some hours and the crystals were filtered off, washed with cold methyl alcohol, dried in vacuo and weighed. To the filtrate water was added, and the clear solution boiled for some time in order to hydrolyse the methyl galactoside formed during hydrolysis. The alcohol was evaporated on the water bath. During the concentration of the watery solution, oily droplets of the sulphates of the bases appeared, which solidified on cooling. The deposit was filtered, taken up in boiling alcohol and the solution evaporated in vacuo. The residue consisting of the sulphates of sphingosine (and dimethylsphingosine) was weighed after having been dried to constant weight in vacuo. The final filtrate was made up to 100 cc. and examined polarimetrically.

Previous experiments had shown us that the results of galactose estimations by the polarimeter agreed with those made by Kjeldahl's gravimetric method.

Experiment I. 1 g. substance gave 0·303 g. esters and 0·620 g. sulphates of bases. Galactose solution: actual rotation measured $= +0·33°$ (mean of six readings) in 2 dm. tube. Whence galactose $= 20·39\,\%$ [see Landolt, 1892, p. 452].

Experiment II. 1 g. substance of a different preparation gave 0·280 g. esters. Galactose solution: actual rotation measured $= +0·34°$ in 2 dm. tube. Whence galactose $= 21·01\,\%$.

d-Galactose was identified in the final solution by means of methylphenylhydrazine. The free mineral acid was neutralised with solid sodium acetate and methylphenylhydrazine was added. After standing at room temperature for some hours, crystals of the hydrazone settled down. They were filtered off and recrystallised from alcohol. The white crystals melted sharply at 191°. The melting point of galactose methylphenylhydrazone is given by Neuberg [1907] as 191° [see also Fränkel, 1910]. From another portion of the hydrolysate, galactose was prepared as such by the usual methods and identified as mucic acid by oxidation with nitric acid. The dry ammonium salt of the mucic acid thus prepared gave on heating a strong pyrrole reaction.

The results are given in the following table (page 610), which shows also for comparison the figures obtained by a similar method by Thierfelder from the hydrolysis of more or less purified galactosides.

It will be seen from these results that the cleavage products obtained on hydrolysis of the galactoside mixture show a close resemblance in their nature, as well as in their quantitative distribution, with those obtainable from the

	Galactoside mixture prepared by the pyridine method		Phrenosin[1] (cerebron)	Kerasin fraction
	I	II		
Nitrogen %	1·68	—	1·76	1·61
Galactose %	20·39	21·01	19·88	19·35
Esters + fatty acid %	30·3	28·0	38·7	29·6
Bases (as sulphates)	62·0	—	50·3	57·2

[1] The figures given represent the mean values calculated by me from Thierfelder's figures.

galactosides isolated by a different method. This result therefore seems to justify the conclusion that the product obtainable from brain by extraction with cold pyridine, consists practically entirely of galactosides. In their preparation any possible decomposition of a preformed complex substance has been carefully avoided by limiting the time of extraction to a minimum and by employing an inert solvent at a low temperature. As, further, the amount obtainable experimentally agrees very well with the theoretical yield, we must assume that the whole of the galactosides exist in the brain in the preformed state.

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Summary.

(1) A new method for the preparation of galactosides from brain by means of pyridine is described.

(2) Evidence is brought forward to show that the galactosides exist in the brain entirely in the preformed condition.

REFERENCES.

Brown, Horace T. and Morris, G. H. (1890), *J. Chem. Soc.* **57**, 57.
Fränkel, S. (1910), *Biochem. Zeitsch.* **26**, 41.
Landolt, H. (1892), *Das Optische Drehungsvermögen*, Braunschweig.
Levene, P. A. and Jacobs, W. A. (1912, 1), *J. Biol. Chem.* **12**, 381.
—— —— (1912, 2), *J. Biol. Chem.* **12**, 389.
—— (1913), *J. Biol. Chem.* **15**, 359.
Neuberg, C. (1907), *Biochem. Zeitsch.* **3**, 531.
Rosenheim, O. and Tebb, M. C. (1910), *J. Physiol.* **40**, Proceedings, i.
—— (1906), *J. Physiol.* **34**, 104.
—— (1913), *Trans. Internat. Congress of Med.* Sect. II. 626.
Smith, J. Lorrain and Mair, W. (1913), *J. Path. Bact.* **17**, 609.
Thierfelder, H. (1890), *Zeitsch. physiol. Chem.* **14**, 209.
—— (1904), *Zeitsch. physiol. Chem.* **43**, 21.
—— (1905), *Zeitsch. physiol. Chem.* **44**, 366.
—— and Kitagawa, F. (1906), *Zeitsch. physiol. Chem.* **49**, 288.
—— (1913), *Zeitsch. physiol. Chem.* **85**, 35.
Thudichum, L. J. W. (1874), *Reports of the Med. Off. of the Privy Council and Local Gvt. Board*, New Series, No. III.
—— (1882), *J. pr. Chem.* **25**, 19.
—— (1901), *Chem. Konstitution d. Gehirns d. Mensch. u. d. Tiere*, Tübingen.

LIX. THE ESTIMATION OF PYRUVIC ACID.

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(*Received Nov. 11th, 1913.*)

Pyruvic acid forms with phenylhydrazine a hydrazone by means of which, as Emil Fischer pointed out, one part of this acid in one thousand parts of water may be detected. The yield of hydrazone however is not quantitative and the attempts made by some authors to estimate pyruvic acid by weighing the phenylhydrazone precipitated have not proved satisfactory. Subsequently nitrophenylhydrazine was used as the precipitating reagent and by this means Neuberg and Karczag [1911] recovered 92 % of pyruvic acid from a 1 % solution, as the nitrophenylhydrazone. In estimating the amount of pyruvic acid in solutions containing 0·1 % and of still lower concentrations this method is however of very little value, since the error introduced by the appreciable solubility of the hydrazone becomes of increasing importance with increasing dilution. An investigation into the action of the tissues on dilute solutions of pyruvic acid which I had undertaken had temporarily to be abandoned since the apparent removal of the acid observed might have been explained by an increase in the amount of hydrazone held in solution, and even when controls of corresponding dilution were used the results were unsatisfactory.

The action of asymmetrical diphenylhydrazine was investigated and was found to present similar difficulties.

If the experimental errors found above were due to the solubility of the hydrazone, the determination of the amount of phenylhydrazine removed from the solution in combination as hydrazone should give satisfactory results.

Estimation of Phenylhydrazine.

Fischer [1878] showed that phenylhydrazine was oxidised by cold dilute Fehling's solution with evolution of nitrogen; benzene and aniline were

formed and cuprous oxide precipitated. Strache and Kitt [1892] estimated the volume of nitrogen liberated and showed that if boiling solutions were used, no aniline was formed and the whole of the nitrogen was liberated in the free state; under these conditions six molecules of cupric oxide were necessary to oxidise two molecules of phenylhydrazine, a mixture of benzene and phenol being obtained. The reaction may be represented as follows:

$$2C_6H_5 . HN . NH_2 + 3O = C_6H_6 + C_6H_5OH + 2N_2 + 2H_2O.$$

The benzene formed during the reaction exerts an appreciable influence on the vapour tension, a difficulty which Strache overcame by saturating the gas both with benzene and with water vapour and introducing the necessary corrections. Strache [1891, 1892] estimated ketones and aldehydes by allowing warm solutions of the carbonyl compound and phenylhydrazine to react and then measuring the excess of phenylhydrazine in the solution by determining the volume of nitrogen evolved when oxidised by boiling Fehling's solution.

The method is not very convenient and it would be preferable to estimate the cuprous oxide formed. By the above method, however, in working with tissue-extracts containing pyruvic acid, any sugar present would react with the boiling Fehling's solution. If, however, the cuprous oxide formed when the excess of phenylhydrazine reacts with Fehling's solution at air temperature be estimated, this difficulty can be obviated.

Experiments were therefore made in order to determine whether the amount of cuprous oxide precipitated by a certain weight of phenylhydrazine was constant.

The Fehling's solution was made up as in Bertrand's method for estimating glucose.

Solution I.	Copper Sulphate crystals 40 grams	per litre.
Solution II.	Rochelle Salt 200 grams Caustic Soda 150 „	per litre.

5 cc. of a solution of phenylhydrazine containing 3·5236 g. in 100 cc. of 50% acetic acid were diluted to 100 cc. with water, and allowed to stand at the ordinary temperature for 30 minutes: in nine experiments, 20 cc. of each of the Fehling's solutions, I and II made up as above, were added to 10 cc. of the diluted phenylhydrazine solution and the mixture allowed to stand at the ordinary temperature for times varying from half an hour to four hours and a half. The cuprous oxide formed was then filtered through a Gooch crucible, dissolved in ferric sulphate solution as in Bertrand's method for the estimation of glucose and the ferrous sulphate produced titrated with deci-normal permanganate solution.

Results.

Time	Cc. N/10 $KMnO_4$ equivalent to Cu_2O formed
30 minutes	6·0
60 ,,	6·05
90 ,,	6·0
150 ,,	6·05
150 ,,	6·0
180 ,,	6·0
210 ,,	6·0
240 ,,	5·95
270 ,,	5·95

The reaction between phenylhydrazine and Fehling's solution appears therefore to reach a definite stage of equilibrium within half an hour at the ordinary temperature, after which no further oxidation proceeds.

In three experiments, the following values were obtained:

1 cc. of N/10 $KMnO_4$ was equivalent to (1) 0·002962 gr. phenylhydrazine,
(2) 0·002962 ,,
(3) 0·002935 ,,

As the mean of these experiments therefore

1 cc. N/10 $KMnO_4$ is equivalent to 0·00295 g. $C_6H_5NH.NH_2$.

It may be mentioned that in filtering the cuprous oxide from the cold Fehling's solution, it is advisable to filter only under a slight difference of pressure, as there is a tendency for the cuprous oxide to pass through the asbestos. If this happens the filtrate should be filtered through a clean Gooch crucible and the two results added together.

Estimation of Pyruvic Acid.

A specimen of Kahlbaum's pyruvic acid was distilled under diminished pressure and the fraction boiling at 77°–78° under a pressure of 15–20 mm. used for the estimation. The method adopted was as follows:

A solution of pyruvic acid was made up containing 1·5228 g. per 100 cc. Quantities of from 2 to 10 cc. of this solution were diluted to about 80 cc., 5 cc. of a solution of phenylhydrazine, approximately 4 %, added, and the mixture made up to 100 cc. and allowed to stand half an hour at the ordinary temperature; 5 cc. of the hydrazine solution were diluted to 100 cc. and allowed to stand for the same time. After half an hour the pyruvic hydrazone which had separated was filtered off and 10 cc. of each filtrate

added to 40 cc. of Fehling's solution. Ten cc. of the control phenylhydrazine solution were similarly treated. The cuprous oxide was estimated as above described.

Thus:

10 cc. phenylhydrazine solution require		6·00 N/10 $KMnO_4$
		6·00 ,, ,,
10 cc. hydrazone filtrate require	...	2·75 ,, ,,
		2·75 ,, ,,

3·25 cc. N/10 $KMnO_4$ are equivalent to $3·25 \times 0·00295$ g. phenylhydrazine.

$$\underset{108}{C_6H_5HN \cdot NH_2} + \underset{88}{CH_3 \cdot CO \cdot COOH} \rightarrow CH_3 \cdot C(N_2HC_6H_5) \cdot COOH.$$

3·25 cc. N/10 $KMnO_4$ are equivalent to $\frac{88 \times 0·00295 \times 3·25}{108}$ g. pyruvic acid, and 10 cc. of the diluted pyruvic solution contained 0·00780 g. pyruvic acid, and the original solution contained 1·556 %.

10 cc. of the diluted solution contained by weight 0·007614 g. and the original solution 1·5228 %.

The following table shows some of the results obtained:

Ten cc. of a solution of pyruvic acid contained:

(*a*) By weight	(*b*) By above method of estimation	Error
2·68 milligrams	2·57 milligrams	− 0·11 mgr.
3·19	2·15	− 1·04
3·57	2·51	− 1·06
5·36	5·14	− 0·22
6·28	6·57	+ 0·29
7·14	7·46	+ 0·32
7·61	7·76	+ 0·15
8·04	7·71	− 0·33
9·42	9·80	+ 0·38
9·58	9·20	+ 0·38
10·71	11·22	+ 0·51
10·71	10·34	− 0·37
11·97	12·31	+ 0·34
12·77	12·67	− 0·10
13·40	13·15	− 0·25

In carrying out the above estimation it is important that the phenylhydrazine solution shall be freshly made up and if it is at all discoloured that the phenylhydrazine shall be freshly distilled.

Influence of Glucose.

Under the conditions above described the presence of glucose does not appear to interfere with the estimation of pyruvic acid. In one experiment,

5 cc. of a solution of phenylhydrazine acetate, 10 cc. of a solution of pyruvic acid and 10 cc. of a 1 % glucose solution were made up to 100 cc. and the pyruvic acid estimated as above, and compared with a solution similarly made up but from which the glucose was omitted.

Residual hydrazine (without glucose) required 4·80 cc. N/10 $KMnO_4$.
,, ,, (with ,,) ,, 4·85 ,, ,,

The method therefore gives satisfactory results in estimating solutions of concentrations above 0·03 %. Below this concentration probably more accurate results would be obtained by using a more dilute solution of permanganate.

The advantages may be summarised as follows:

(1) It is easily carried out; the whole estimation can be done in little more than an hour.

(2) It gives a greater degree of accuracy than the unsatisfactory method of gravimetric estimation at present in use.

(3) The presence of glucose does not interfere with the estimation.

(4) The method promises to be of general value for the estimation of carbonyl compounds and also for measuring the rate of interaction of these compounds with phenylhydrazine.

REFERENCES.

Fischer, E. (1878), *Annalen*, **190**, 101.
Neuberg and Karczag (1911), *Biochem. Zeitsch.* **36**, 63.
Strache (1891; 1892), *Monatsh.* **12**, 524; **13**, 299.
—— and Kitt (1892), *Monatsh.* **13**, 316.

LX. NOTE ON ISOCHOLESTEROL, COPROSTEROL AND THE CLASSIFICATION OF THE STEROLS.

By CHARLES DORÉE.

(*Received Nov. 9th, 1913.*)

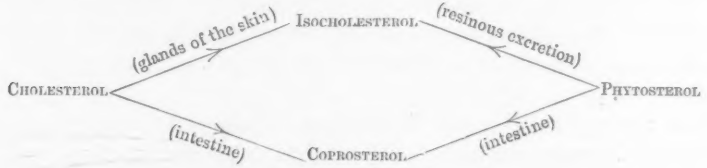
Isocholesterol was discovered by Schulze [1873] in the fat extracted from raw sheep's wool with ether. The fat is derived from the secretion of the fatty glands surrounding the hair follicle in the skin. For many years the individuality, and even the existence of isocholesterol, were questioned, as many workers, including the present writer, were unable to obtain it. In reply to criticisms of Darmstädter and Lifschütz [1898], Schulze [1898] repeated his original experiments and subsequently Moreschi [1910] obtained the substance from wool fat and fully confirmed the observations of Schulze. In 1910 additional interest was given to the question by the discovery of isocholesterol in a plant product, the so-called South African rubber, which consists of the coagulated latex of various species of Euphorbiaciae. The latex contains about 6 per cent. of rubber and 70 per cent. of resin. The isocholesterol was found as a constituent of the latter (excretory) product [Cohen, 1908]. A careful comparison of the substance with a specimen of the original isocholesterol supplied by Schulze was made by Cohen, and in my opinion no doubt can be entertained as to the identity of the two products and consequently of the recognition of isocholesterol as a definite member of the sterol group.

Coprosterol occurs normally in the faeces of men on ordinary diets, and in those of animals, in place of cholesterol, when diets rich in cholesterol, e.g. raw brain, are given [Dorée and Gardner, 1908]. It is no doubt produced from the cholesterol of the food by changes taking place in the intestine. König and Schluckebier [1908] have stated also that it appears in the excrement of animals which have been kept on diets rich in phytosterol such as peas, maize, coco-nut cake, etc. [Cp., however, Kusumoto, 1908; Dorée and Gardner, 1909.]

The recognition of isocholesterol as a definite member of the sterol group leads me again to point out that while isocholesterol and coprosterol, in their

mode of occurrence and properties, closely resemble one another, they differ in these respects markedly from all the other sterols [Dorée, 1909, 1]. Hitherto the sterols have been classified roughly as zoo- or phyto-sterols according as they were obtained from animal or vegetable sources respectively. But in order to emphasise the special properties and relationships of isocholesterol and coprosterol it is now suggested that a third class should be formed to include them and any other similar substances that may be discovered, and that the terms zoo- and phyto-sterols should be limited to a somewhat more exact definition. Seeing that coprosterol and isocholesterol undoubtedly stand in a close relationship to cholesterol and phytosterol, and that they appear to form a connecting link between these characteristic constituents of animal and vegetable protoplasm, they may be classified as "natural derived sterols" or *metasterols*.

The metasterols occur in the excretions and secretions of animals (intestinal canal, glands of the skin) and of plants (resin). They are never found as constituents of animal or vegetable protoplasm. The connection between them and the other sterols from a biochemical standpoint is shown in the following scheme.



The metasterols are monatomic alcohols of high molecular weight. Unlike the other sterols they (*a*) are saturated towards bromine and hydrogen [Moreschi, 1910], (*b*) do not crystallise from alcohol in plates, (*c*) give the colour tests of Liebermann and Salkowski in a modified way, (*d*) have practically no anti-toxic action towards haemolytic poisons [Hausman, 1905], (*e*) are dextro-rotatory.

It is probable that spongosterol [Henze, 1908], which has no anti-toxic power and apparently is a saturated alcohol, will fall into this class. The position of stigmasterol, $C_{30}H_{48}O$ [Windaus and Hauth, 1906], and brassicasterol, $C_{28}H_{46}O$ [Windaus and Welsch, 1909], found, together with large proportions of ordinary phytosterol in Calabar beans and rape seed respectively, is uncertain.

With the formation of this class it is now proposed to limit the terms zoo- and phyto-sterol to sterols which are found as tissue constituents of

animals and plants respectively. Cohen [1908], in the light of his discovery of isocholesterol as a plant product, has stated that the distinction between zoo- and phyto-sterols can no longer be maintained. But with the proper recognition of isocholesterol as a derivative produced from zoo- or phyto-sterols by metabolic changes, there is now every reason for maintaining it. For in spite of a great number of researches (Hauth [1907] quotes some 50 papers and many more have since appeared), no substance resembling cholesterol has ever been obtained from vegetable protoplasm or one resembling phytosterol from animal protoplasm; and this in spite of the fact that herbivorous animals take in phytosterol with their food and the carnivorous plants ingest a cholesterol. If phytosterol taken with the food is used by the animal in building up its tissues, it must first be converted into cholesterol. Furthermore a zoosterol is an invariable constituent of all animal organs and tissues so far examined from Chordata to Coelenterata [Dorée, 1909, 1; Welsch, 1909], and a phytosterol seems similarly to be contained in the tissues of plants of all orders. So fundamental is this distinction considered to be that it has become an important criterion in chemical technology for deciding the animal or vegetable origin of various natural substances. Thus Lewkowitsch [1913] defines these products to be of animal or vegetable origin respectively according to the presence of a zoo- or phyto-sterol in the unsaponifiable portion. [Cf. also the 'phytosterol acetate test.']

The zoo- and phyto-sterols will therefore include such sterols as are found entering into the composition of animal and vegetable protoplasm respectively. Speaking only of those whose properties have been carefully examined (for zoosterols see Dorée [1909, 1] and Welsch [1909]; for phytosterols see Hauth [1907]) the zoo- and phyto-sterols may be held to include "a number of secondary, monatomic alcohols, chiefly $C_{27}H_{46}O$, containing one ethylene linking in the molecule. They crystallise from alcohol in plates. Their benzoates melt at about 145° and show the phenomena of liquid crystals. They are all laevo-rotatory and have a powerful anti-haemolytic action towards saponine, etc." Their constant presence in the tissues is in part due to the necessity for the protection of the cells from such toxic substances: their occurrence in the fluids and secretions shows that they are continually being metabolised and, it is believed, conserved by the organism [Dorée and Gardner, 1909].

A summary of the properties of the best known sterols and their derivatives is given in the following table:

	M.p.	$[\alpha]_D$	Acetate m.p.	Benzoate m.p.	Dibromide m.p.	Occurrence
(*a*) Zoosterols:						
Cholesterol	147°	−37°	114°	145°	123°	Universally distributed in the animal kingdom.
Bombicesterol	148°	−35°	129°	146°	111°	In *Bombyx mori* [Menozzi and Moreschi, 1908].
Clionasterol	138°	−37°	133°	143°	114°	In *Cliona celata* [Dorée, 1909, 1].
(*b*) Phytosterols:						
Phytosterol	137°	−34°[1]	127°	146°	98°	Universally distributed in the Phanerogams.
(*c*) Metasterols:						
Isocholesterol	137°	+60°	134°	194°	None	Wool fat; resin of Euphorbiaciae.
Coprosterol	100°	+24°	88°	122°	None	Excrement of animals.

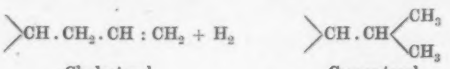
[1] In ether solution; others in chloroform.

Of the chemical relationship existing between cholesterol, phytosterol and the metasterols little is known. On a milk diet, during which bacterial change in the intestine is reduced to a minimum, cholesterol [Müller, 1900] and phytosterol [Windaus, 1908] are not converted to coprosterol. Normally in the case of men, and on diets rich in sterols in the case of animals, coprosterol is produced. Analyses, to which however no great weight can be assigned, seemed to indicate that the coprosterol molecule contained two atoms of hydrogen more than that of cholesterol, and it has been thought that coprosterol was dihydrocholesterol produced by bacterial reduction. Dihydrocholesterol artificially produced [Willstätter and Mayer, 1908] is not however identical with coprosterol, although very similar in its properties as will be seen from the following table:

	M.p.	$[\alpha]_D$	Acetate m.p.	Benzoate m.p.	Ketone m.p.	Observer
Dihydrocholesterol	142°	+29°	111°	155°	128°	Willstätter.
Coprosterol	100°	+24°	88°	122°	63°	Dorée.
Isocholesterol	137°	+60°	134°	194°	?	Cohen.

Observations on a large number of cholesterol and phytosterol derivatives have shown that any modification of the ethylene linking causes a change in the optical rotatory power from negative to positive and at the same time the antihaemolytic function is abolished or reduced to a minimum [Hausman, 1905]. The presumption therefore is strong that it is the side chain containing the double linking in cholesterol that is modified to produce coprosterol. The change however is more than one of simple reduction. The only other explanation that has been offered, based upon the behaviour of a peculiar carbazole derivative of coprostanone [Dorée, 1909, 2], is that the

bacterial action might simultaneously bring about reduction and rearrangement of the unsaturated side chain, producing two methyl groupings, thus:



Cholesterol Coprosterol

Attempts have been made to solve the problem by studying the oxidation of coprosterol and cholesterol in the hope of obtaining, among the products, a derivative common to both. Coprosterol is difficult to obtain in any quantity, and considering the similarity between coprosterol and isocholesterol and the fact that the raw material from which isocholesterol is obtained is a commercial product, I decided two years ago to work up a large quantity of wool fat in the hope of preparing sufficient isocholesterol to enable a thorough examination of that substance to be made. Quantities of 2 to 3 kilos of wool fat from various sources were saponified under pressure by the method of Lewkowitsch and the mass extracted with ether in the usual way. The unsaponifiable residue was then fractionated in alcohol, acetone, methyl alcohol, etc. None of the fractions appeared to contain isocholesterol and they were therefore severally benzoylated. A number of crystalline benzoates were obtained, but none melting higher than 145°. A quantity of wool fat treated by the method of Darmstädter and Lifschütz also gave a negative result. An explanation of these results may be found in the observation of Cohen [1908] that isocholesterol undergoes a change of the nature of autoxidation on keeping. The somewhat drastic treatments to which commercial wool fat is subjected may therefore have destroyed the isocholesterol. The best method of obtaining it would seem to be to extract fresh clipped raw wool with ether, or to employ the resin of South African rubber.

The expenses of these experiments were covered by a grant from the Government Grant Committee of the Royal Society for which I desire to express my thanks.

REFERENCES.

Cohen (1908), *Arch. Pharm.* **246**, 518, 592.
Darmstädter and Lifschütz (1898), *Ber.* **31**, 98, 1126.
Dorée (1909, 1), *Biochem. J.* **4**, 73.
—— (1909, 2), *J. Chem. Soc.* **95**, 655.
—— and Gardner (1908), *Proc. Roy. Soc.* B. **80**, 230.
—— —— (1909), *Proc. Roy. Soc.* B, **81**, 109.
Hausman (1905), *Beiträge*, **6**, 567.
Hauth (1907), *Zur Kenntnis der Phytosterine*, Diss. Freiburg.
Henze (1908), *Zeitsch. physiol. Chem.* **55**, 427.

König and Schluckebier (1908), *Zeitsch. Nahr. Genussm.* **15**, 654.
Kusumoto (1908), *Biochem. Zeitsch.* **142**, 407.
Lewkowitsch (1913), *The Chemical Technology and Analysis of the Oils, Fats and Waxes*, 3rd Ed. Macmillan and Co.
Menozzi and Moreschi (1908), *Atti R. Accad. Lincei* [v], **17**, ii, 95.
Moreschi (1910), *Atti R. Accad. Lincei* [v], **19**, ii, 53–57.
Müller (1900), *Zeitsch. physiol. Chem.* **29**, 129.
Schulze (1873), *J. pr. Chem.* **7**, 169.
—— (1898), *Ber.* **31**, 1200.
Welsch (1909), *Vorkommen der Sterine in Tier. u. Pflanzenreich*, Diss. Freiburg.
Willstätter and Mayer (1908), *Ber.* **41**, 2199.
Windaus (1908), *Arch. Pharm.* **246**, 121.
—— (1911), *Die Sterine, Biochemisches Handlexikon* (Abderhalden), **3**, 268.
—— and Hauth (1906), *Ber.* **39**, 4378.
—— and Welsch (1909), *Ber.* **42**, 612.

LXI. THE HYDROLYSIS OF GLYCOGEN BY DIASTATIC ENZYMES. II. THE INFLUENCE OF SALTS ON THE RATE OF HYDROLYSIS. (Preliminary Communication.)

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(*Received Nov. 17th, 1913.*)

In a recent communication [Norris, 1912] it was pointed out that samples of glycogen prepared from different animals were hydrolysed at different rates by pancreatic amylase. In view, however, of the fact that these preparations contained varying amounts of salts it seemed desirable to examine how far this might influence the rate of hydrolysis, and a quantitative study has therefore been made of the action of neutral salts on diastatic action. The results quickly showed that the experiments referred to were in no way invalidated by the varying salt content, the latter in every case being sufficient to produce the maximum degree of hydrolysis and not great enough to cause any inhibition.

The experiments have however been continued in the hope of finding some explanation of the manner in which salts exert their influence.

It has been known for some time that a dialysed amylase solution when added to a starch solution free from salts produces but little hydrolysis, and that on adding certain salts in small quantities the activity of the enzyme is restored. In spite of a considerable amount of work on this point, the explanation of this fact is not by any means clear. It is agreed that the most active salts are those of the halogen acids. With regard to sulphates, however, very divergent results have been obtained. Cole [1906, 1], for example, states that sulphates accelerate the action while Grützner [1902] maintains that magnesium and sodium sulphate are "specific poisons" for the ferment. In this connection however it must be pointed out that in one at least of Cole's experiments the addition of sulphate produced no acceleration although the concentration of the salt was similar to that used in previous experiments in which an acceleration had been obtained. These and other divergencies

may perhaps be partly explained by one of the following reasons. Firstly, the source and method of preparation of the enzyme has differed with nearly every worker. Hence while some investigators have employed solutions containing only traces of proteins, in other cases these have been present in considerable quantity. Again, in the few cases where the action has been followed quantitatively, the diastatic activity has been estimated either by Roberts' achromic point method [Roberts, 1891] or by the method of Wohlgemuth [1908, 1], and it has been shown by Evans [1912, 1] that neither of these is satisfactory. Finally, in some cases, the enzyme and starch solution employed have been by no means free from salts, the controls all showing a marked degree of hydrolysis, that is to say the observed effect was really due to a mixture of salts and not alone to the particular salt under investigation.

In the following experiments glycogen has been employed instead of starch, while the enzyme has consisted of an extract of pigs' pancreas.

Preparation of glycogen. This was obtained from dogs' liver by Pflüger's method. The crude glycogen was purified by repeated precipitation of its solution by alcohol and was finally dialysed for a week, the last three days' dialysis being against running distilled water.

Preparation of enzyme. This consisted of a Buchner extract of pigs' pancreas which was dialysed for three days, in the course of which a certain amount of protein usually separated out. The dialysed extract was then filtered till perfectly clear and diluted from ten to twenty times with distilled water.

Experimental methods. The following may be described as typical of the method employed.

A 1 per cent. or 2 per cent. solution of glycogen was as a rule used and to this was added a suitable concentration of the salt under investigation. The mixture was then brought to a temperature of 37° in a thermostat and the enzyme added. After 15 minutes and 30 minutes hydrolysis, 20 cc. of the mixture were removed and the sugar immediately estimated by Bertrand's method. The sugar solution was added directly to the alkaline copper mixture and hence the hydrolytic action stopped instantaneously. The strength of enzyme used was adjusted so that the readings taken fell on the linear portion of the hydrolysis curve. [See Evans, 1912, 2 and Norris, 1912.] The salts employed were nearly all Kahlbaum's "for analysis with certificate of guarantee."

Effect of dialysis on glycogen hydrolysis.

As in the case of starch the result of dialysis of both enzyme and glycogen resulted in almost complete inactivation. Similarly the hydrolytic power was again restored by the addition of certain salts.

Influence of salts.

Under this heading neutral salts only are considered. The rate of hydrolysis is of course greatly influenced by any change in the reaction of the medium, but this point has been dealt with in a previous communication [Norris, 1912].

Hydrogen ion determinations made on glycogen solutions containing varying concentrations of sodium chloride showed that the reaction of the medium was not altered by the presence of this salt in the concentrations employed, hence the accelerating effect of sodium chloride is not due to this cause.

Sodium chloride.

A series of mixtures was made up each containing 1 per cent. glycogen and a concentration of sodium chloride ranging from zero to 0·003 N. These were in turn incubated with 1 cc. of a dilute enzyme preparation and hydrolysis allowed to proceed for 15 minutes. The sugar in 20 cc. of each solution was then estimated.

The results are shown in Fig. 1 where the abscissae represent the cc. of 0·1 N NaCl in 100 cc. of the mixture and the ordinates the cc. of $KMnO_4$ used in the sugar estimation.

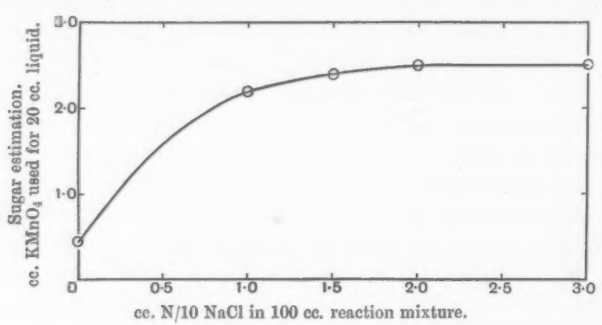

Fig. 1.

The results show that there is a rapid increase in the hydrolysis with increasing NaCl content until the latter reaches a concentration of about 0·002 N (0·012 per cent.). This value is in close agreement with Cole's results with starch and ptyalin.

On further addition of salt there is at first no change in rate, but with high concentration a slight retardation may be produced.

An investigation was next undertaken to decide whether the value of this optimum concentration (0·002 N) would be changed by alteration in the concentration of either glycogen or enzyme. On this point results have been somewhat contradictory but it seems probable that the glycogen concentration has but little influence on the amount of salt required to produce the maximum rate of hydrolysis. The optimum salt content for 2 per cent. glycogen has usually been about 0·002 N, that is to say the same as for 1 per cent. glycogen.

On increasing the amount of enzyme, however, a higher concentration of NaCl is usually required. This is in agreement with the results of Cole [1906, 1] and Starkenstein [1910]. The latter, working with starch, states that the amount of NaCl necessary to give the maximum rate of hydrolysis varies *directly* with the concentration of enzyme and has even based on this a method for the estimation of diastase in animal organs [Starkenstein, 1912]. The results of my experiments do not point to such a simple relationship, though as already stated more NaCl is usually required with increased concentration of enzyme. It must be remembered that it is usually in the enzyme that impurities such as proteins etc. will be found and the latter may begin to play an important part when the amount of enzyme present is not small.

The following table shows the results of one experiment in which the concentration of enzyme was twice that usually employed.

TABLE I.

Determination of NaCl optimum with 1 per cent. glycogen and high enzyme concentration.

Experiment	Concentration of NaCl	Percentage hydrolysis	
		15 mins.	30 mins.
A	0	5·5	9·20
B	0·002 N	15·1	26·5
C	0·004 N	16·5	28·0
D	0·006 N	18·3	29·8
E	0·01 N	18·3	29·5

In this case therefore by doubling the concentration of enzyme the optimum salt concentration was raised from 0·002 N to 0·006 N, that is to say three times.

In other experiments however very much lower results were obtained and there seems to be some unknown factor concerned. The point is still under investigation.

Comparison of sodium chloride with other salts.

Chlorides of different metals.

Table II shows the accelerating effect of the chlorides of sodium, potassium, calcium, barium and magnesium.

These were added in insufficient quantity to produce the optimum rate of hydrolysis, so that any variation in their accelerating power could be detected.

TABLE II.

Accelerating power of various chlorides. 1 *per cent. glycogen.*
Concentration of salt = 0·0005 *N.*

		Percentage hydrolysis	
	Salt	15 mins.	30 mins.
	NaCl	10·83	18·76
	KCl	10·61	18·8
	$CaCl_2$	10·70	—
	$BaCl_2$	10·61	17·6
	$MgCl_2$	10·61	18·52
Control	0	0·68	1·14

All the above salts are therefore of equal accelerating power, that is to say the kation exerts practically no influence on the reaction. This is in agreement with the results of Starkenstein [1910]. Cole [1906, 1] considered that the anion accelerated while the kation depressed the action. If the latter were correct, however, one would expect the chlorides containing a divalent kation to be less active than sodium or potassium chloride and as shown this is not the case. The view that the anion is the more important factor is further strengthened by a comparison of the accelerating power of chlorides, bromides and iodides. The results given in Table III confirm those of previous investigators, namely, that the acceleration decreases in the order given, the drop from bromide to iodide being much greater than that from chloride to bromide.

TABLE III.

Comparison of KCl, KBr and KI. (*Concentration of salt* = 0·0005 *N.*)

Salt	Percentage hydrolysis 15 mins.	30 mins.
KCl	10·61	18·80
KBr	8·31	15·60
KI	2·5	3·70
Control	0·68	1·40

Influence of sulphates.

The influence of three sulphates has been investigated but it has been found that they have no accelerating power at all; these results are therefore in agreement with those of Wohlgemuth [1908, 2] but in opposition to those of Cole [1906, 1]. The latter however found that sulphates were much less effective than the halogen compounds. These results are of some interest, as one would expect if the anion were the factor concerned, that the divalent anion would be extremely potent. The situation is complicated, however, as pointed out by Cole, by the fact that sodium sulphate in moderate dilutions chiefly dissociates into Na^+ and $NaSO_4^-$ in which case the anion is monovalent.

On the other hand it has not been found that sulphates have any depressing action [cf. Grützner, 1902], nor do they hinder the acceleration produced by NaCl etc.

TABLE IV.

Influence of sulphates. 1 *per cent. glycogen.*

	Salt	Concentration of salt	Percentage hydrolysis 15 mins.	30 mins.
1.	0	Control	1·60	5·04
	Na_2SO_4	0·002 N	1·65	5·0
	Na_2SO_4	0·01 N	1·55	5·0
	$MgSO_4$	0·001 N	1·60	—
2.	$MgSO_4$ + NaCl	Each 0·001 N	9·10	16·04
	NaCl	0·001 N	9·20	16·10

For the sake of comparison the results obtained with different salts have been collected in Table V.

TABLE V.

Comparison of various salts. 1 *per cent. glycogen containing* 0·0005 *N salt.*

Salt	Percentage hydrolysis 15 mins.	30 mins.	Remarks
0 (Control)	0·68	1·14	
NaCl	10·83	18·76	Halogen salts. Both ions monovalent.
KCl	10·61	18·8	
KBr	8·31	15·6	
KI	2·5	3·7	
$CaCl_2$	10·70	—	Halogen salts with divalent kation.
$BaCl_2$	10·61	17·6	
$MgCl_2$	10·61	18·5	
KNO_3	2·75	5·08	Both ions monovalent.
$La(NO_2)_3$	2·75	4·94	Trivalent kation.
Na_2SO_4	0·55	1·0	Divalent anion.
K_2SO_4	0·64	1·20	,, ,,
$MgSO_4$	0·70	—	Both ions divalent.

It will be seen from the results tabulated above that the only salts of those tried which have a powerful accelerating action are those of the halogen acids, although nitrates have a small influence. It is also clear that the anion is much more concerned in the reaction than is the kation. Further it is probable that the action of the salts is chiefly confined to the enzyme, for it has been shown by Cole [1906, 2] that in the action of invertase, where the substrate is not a colloidal solution, salts have again a powerful influence. In this case, however, the action is reversed, that is to say, the hydrolysis is retarded by chlorides.

If the function of the anion be simply to alter the charge on the enzyme, it is difficult to understand why sulphates have no accelerating effect, for sulphates discharge a ferric hydroxide solution much more readily than chlorides. The fact that the number of salts producing an acceleration is so restricted, however, points to some other explanation.

In the meantime it seems desirable to examine separately the effect of salts on the glycogen and enzyme respectively from the point of view of adsorption and charge, and experiments on these lines are in progress but are not as yet sufficiently advanced to furnish an explanation. The action of a further series of salts is also being investigated.

Summary.

(1) A dialysed glycogenase (pancreatic) solution has practically no hydrolysing action when added to a dialysed glycogen solution.

(2) The activity of the enzyme is restored by the addition of small quantities of certain salts.

(3) The most powerful of these are the salts of the halogen acids, the activity diminishing in the order chlorides, bromides, iodides; nitrates have also a slight accelerating action.

(4) Sulphates do not restore the activity of a dialysed enzyme solution, neither are they inhibitors [cf. Grützner, 1902].

(5) The concentration of salt required to produce a maximum degree of hydrolysis rises with increasing enzyme concentration but appears to be independent of the glycogen concentration within the limits tried.

(6) The anion is probably the part of the salt concerned in the acceleration, the nature of the kation (valency) having no influence.

REFERENCES.

Cole (1906, 1), *J. Physiol.* **30**, 202.
—— (1906, 2), *J. Physiol.* **30**, 281.
Evans (1912, 1), *J. Physiol.* **44**, 220.
—— (1912, 2), *J. Physiol.* **44**, 191.
Grützner (1902), *Pflüger's Archiv*, **91**, 195.
Norris (1912), *Biochem. J.* **7**, 26.
Roberts (1891), *Digestion and Diet*, London, p. 68.
Starkenstein (1910), *Biochem. Zeitsch.* **24**, 210.
—— (1912), *Biochem. Zeitsch.* **47**, 300.
Wohlgemuth (1908, 1), *Biochem. Zeitsch.* **9**, 1.
—— (1908, 2), *Biochem. Zeitsch.* **9**, 10.

LXII. THE ENZYMATIC FORMATION OF POLYSACCHARIDES BY YEAST PREPARATIONS.

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(*Received Nov. 17th, 1913.*)

Two reasons led to the institution of the following experiments. In an earlier paper [1904] the authors showed that the carbon dioxide evolved in the alcoholic fermentation of sugars by yeast juice was not equivalent to the sugar which disappeared from the solution, and ascribed this fact to the production of a hydrolysable compound of low reducing power. It was subsequently found that in alcoholic fermentation by yeast preparations a certain amount of hexosephosphate is formed, which has a lower reducing power than the sugar (about 75 per cent.) from which it is formed and would therefore in part account for the phenomenon.

In the second place it appears to follow from the authors' equations of fermentation [1908] that in the normal fermentation both of fructose and glucose half the sugar passes through the form of hexosephosphate, which is then hydrolysed. Since these two hexoses appear to yield the same hexosephosphate it would be expected that as the fermentation proceeded fructose and glucose alike would be partially converted into the same product of hydrolysis, and the rotations of their solutions should therefore tend to approximate to each other. The exact nature of the substance produced along with phosphoric acid by the hydrolysis of hexosephosphate in yeast juice is not definitely known, but all the evidence points towards its being fructose. Hence we should expect in all fermentations of glucose by yeast or yeast preparations a progressive conversion of glucose into fructose [compare Slator, 1911]. This however has not been observed and experiments were therefore made on the subject, especially with the object of ascertaining whether the product of hydrolysis of the hexosephosphate underwent any secondary change, such as condensation to a polysaccharide.

The present experiments show that both from glucose and fructose one or more dextrorotatory polysaccharides are produced during alcoholic

fermentation by yeast preparations. The previous conclusion of the authors is therefore confirmed, but it is not yet settled whether the polysaccharide formation takes place at the expense of the glucose and fructose themselves or occurs indirectly as the result of the action of some enzyme on the product of hydrolysis of the hexosephosphate. Further investigations on this point are in progress.

It is well known that living yeast forms glycogen when brought into excess of sugar solution [see Pavy and Bywaters, 1907], and the behaviour of yeast preparations therefore indicates that the enzymes involved in this synthesis are probably, at least to some extent, still present and active. The isolation of a substance having the qualitative reactions of glycogen is a further confirmation of the observation of Cremer [1899] who found that in yeast juice free from glycogen a substance was slowly formed in the presence of sugar which gave the characteristic glycogen reactions.

Experimental.

Experiment 1. Three lots of 100 cc. maceration juice (from Schroder's dried Münchener yeast) were incubated at 25° with toluene until they had attained the temperature of the bath. To Nos. 1 and 2 were then added 25 cc. of a 40 per cent. solution of glucose and the evolution of carbon dioxide observed. At the same time No. 3 was boiled and cooled, and 25 cc. of the same glucose solution added.

In Nos. 1 and 2 a maximum rate of 21·6 cc. per 2 minutes was slowly attained, which then rapidly diminished until in 52 minutes a constant rate of 2·8 cc. per 2 minutes was reached. The initial high rate was due to the presence of free phosphate in the maceration juice, which was converted into hexosephosphate. During this period the total gas evolved was 293·6 cc. at room temperature and pressure. After 52 minutes No. 2 was boiled, whilst the fermentation in No. 1 was allowed to proceed for 17 hours 38 minutes, during which time 1458 cc. of CO_2 had been evolved. No. 1 was then boiled. The contents of all three flasks were then filtered, and the free phosphate estimated in an aliquot portion of each. The amount of glucose in each was determined by precipitating the proteins in aliquot portions with Patein's mercuric nitrate solution and estimating the glucose by means of Pavy's method.

The treatment with mercuric nitrate precipitates the hexosephosphate, so that in order to determine the amount of sugar used up allowance must be made for the quantity bound up in the form of hexosephosphate. This is

readily done since it has given rise to the CO_2 equivalent to the free phosphate present at the beginning, and can therefore be determined by subtracting the carbonic acid corresponding with the constant rate of fermentation from the total actually evolved, in the manner frequently described before.

Free phosphate in No. 3 = 1·110 g., in No. 2 = 0·130 g. and in No. 1 = 0·127 g. $Mg_2P_2O_7$, showing that the same quantity of sugar is still bound up as hexosephosphate in No. 1 as in No. 2.

CO_2 evolved up to the time when No. 2 was boiled (after 52 mins. fermentation)	293·6 cc.
Rate = 2·8 × 26	72·8 „
Equivalent of phosphate	220·8 cc. or 201 cc. at N.T.P., i.e. 0·394 g.

This corresponds therefore to 0·79 g. glucose.

The amount of glucose converted into hexosephosphate may also be determined from the phosphate combined during the experiment. Phosphate bound up in No. 2 = 1·110 − 0·130 = 0·980 g. $Mg_2P_2O_7$; equivalent therefore to $0{\cdot}980 \times \frac{180}{222} = 0{\cdot}795$ g. glucose.

The tables show the amount of glucose which cannot be accounted for as CO_2 and alcohol or as hexosephosphate, the glucose originally present being obtained from No. 3 by analysis.

Flask (2).

CO_2 evolved = 293·6 cc. or 267 cc. at N.T.P. = 0·53 g. equivalent to		1·06 g. glucose.
Glucose bound up as hexosephosphate		0·79 g. „
Total accounted for		1·85 g. „
Original glucose	9·98 g.	
Final glucose	8·15 g.	
Actual loss	1·83 g.	

No disappearance of glucose was observed.

Flask (1).

CO_2 evolved = 1458 cc. or 1339 cc. at N.T.P. or 2·634 g. equivalent to		5·27 g. glucose.
Glucose bound up as hexosephosphate		0·79 g. „
Total accounted for		6·06 g. „
Original glucose	9·98 g.	
Final glucose	0·91 g.	
Actual loss	9·07 g.	

Thus 9·07 − 6·06 = 3·01 g. of glucose have disappeared.

The ratio between the reducing power and the optical rotation was determined in each mixture after the treatment with Patein's solution in

order to see if any active substance other than sugar were present. For the sake of convenience the rotation observed in a 400 mm. tube is compared with the reducing power determined by Pavy's method expressed as grams of glucose in 100 cc.

With pure glucose this ratio $\frac{\text{Rotation in 400 mm. tube}}{\text{Reduction (g. glucose per 100 cc.)}} = +2{\cdot}05$, whilst with pure fructose it is $-4{\cdot}03$.

These ratios were found to be

(1) $+\frac{2{\cdot}149}{0{\cdot}120} = +17{\cdot}91.$

(2) $+\frac{2{\cdot}224}{1{\cdot}08} = +2{\cdot}06.$

(3) $+\frac{2{\cdot}843}{1{\cdot}32} = +2{\cdot}15.$

It is thus seen that in No. 1 some substance is present which has a much greater dextrorotatory power than has glucose, whereas in No. 2 all the rotatory power may be accounted for by the quantity of glucose present.

Experiment 2. A similar experiment was carried out with fructose (Kahlbaum), the following mixtures being employed:

(1) 100 cc. maceration juice + 25 cc. 40 per cent. fructose + toluene.
(2) 100 cc. „ „ + 25 cc. 40 per cent. „ „
(3) 100 cc. „ „ + 25 cc. water + toluene.
(4) 100 cc. „ „ + 25 cc. „ „

The juice in each case was kept in the bath until the temperature was attained, and the fructose and water then added. Nos. 1 and 3 were boiled immediately, whilst the others were incubated and the fermentation observed. In No. 2, a high phosphate rate of 53 cc. per 5 minutes was rapidly reached which then decreased as usual to a constant rate of 7·5 cc. per 5 minutes; at the end of 17 hours both Nos. 2 and 4 were boiled. During the first 70 minutes No. 2 had given off 352·5 cc. of carbon dioxide, the amount due to the phosphate thus being $352{\cdot}5 - 14 \times 7{\cdot}5 = 352{\cdot}5 - 105 = 247{\cdot}5$ cc.; the total evolved in the 17 hours was 1179·5 cc. at 762·8 mm. and 15°. No. 4 showed no fermentation at all.

A portion of each of the four filtered mixtures was treated with Patein's solution as in the last experiment, and the reducing power and rotations determined in an aliquot portion of the filtrate. The figures given are calculated for the total volume of the juice.

	1. Original mixture	2. After 17 hrs.	3. Original juice alone	4. Juice incubated alone
Rotation in 400 mm. tube	−30·81°	+4·65°	−0·061°	−0·066°
Total sugar as g. glucose	9·31	1·10	0	0

Loss of sugar = (1) − (2) = 8·21 g.

CO_2 evolved = 1179·5 cc. at 762·8 mm. and 15° = 2·08 g.

CO_2 corresponding to fructose bound up as hexosephosphate = 247·5 cc. at 762·8 mm. and 15° = 0·41 g.

Total sugar accounted for as CO_2 and hexosephosphate therefore

$$= 2 \times 2{\cdot}49 = 4{\cdot}98 \text{ g.}$$

Sugar disappeared = 8·21 − 4·98 = 3·23 g.

The ratios of rotation to reduction expressed as before were also determined in the solution after treatment with Patein's solution and were found to be (1) − 4·14, (2) + 5·28; pure fructose = − 4·03.

It is thus seen that in this experiment a substance having a high dextrorotation was formed, so that although all the fructose was not used up, the mixture had changed in rotation from laevorotatory to dextrorotatory, whilst a much larger proportion of the original fructose had disappeared than could be attributed to the fermentation.

In the solutions before treatment with Patein's solution the free phosphate was estimated and was found to be:

(1) 1·203 g. $Mg_2P_2O_7$.
(2) 0·174 g. „
(3) 1·210 g. „
(4) 1·208 g. „

From this it is seen that the phosphate and hence an equivalent portion of the sugar was still bound up as hexosephosphate at the end of the experiment (No. 2). These numbers serve as before as a check on the quantity of sugar which has been converted to hexosephosphate, viz. that amount corresponding to

$1{\cdot}203 - 0{\cdot}174 = 1{\cdot}029$ g. $Mg_2P_2O_7$ or $1{\cdot}029 \times \frac{180}{222}$ fructose = 0·834.

Fructose calculated from equivalent of CO_2 as above = 0·41 × 2 = 0·82 g.

The mixtures before treatment with Patein's solution were tested with iodine solution; Nos. 1, 3, and 4 gave no colouration, whereas No. 2 gave a deep reddish brown colouration.

A portion of No. 2 treated with three volumes of alcohol gave a white precipitate, which was redissolved in water and again precipitated with alcohol. This last precipitate gave an opalescent solution in water which was precipitated by saturation with ammonium sulphate and gave a red colouration with iodine. The only difference which could be seen from the behaviour of glycogen was that it gave a somewhat different red colour with iodine.

The other solutions Nos. 1, 3, and 4 gave slight precipitates with alcohol, the aqueous solutions of which gave however no colouration with iodine.

It is thus seen that during the fermentation of fructose by maceration juice a dextrorotatory, glycogen-like substance is formed.

These results appear to us as already indicated to throw some light on the cause of the difference which exists between sugar fermented and carbon dioxide evolved, not only in the case of yeast preparations but also in that of living yeast. Euler and his colleagues in recent papers have argued from the existence of this difference between the amount of sugar actually removed by living yeast from a glucose solution and the amount equivalent to the CO_2 evolved, which he terms $\Delta - C$, that the hexose requires to undergo some change which renders it directly fermentable and that the difference $\Delta - C$ represents the amount which is in this intermediate condition. [Euler and Johannson, 1912; Euler and Berggren, 1912.] There seems however to be no good reason to suppose that Euler and Johannson's $\Delta - C$ cannot be accounted for by the well-known formation of glycogen which has been shown by Pavy and Bywaters [1907] to be of the order of magnitude required.

In Euler and Berggren's experiments on the effect of yeast extract in increasing both rate of fermentation and $\Delta - C$ [1912], no counts of yeast cells before and after the experiments were made. As the earliest observations were made after an hour at 15°–18° and the experiments in some cases extended to over six hours (1 g. pressed yeast in 25 cc. of solution), the possibility of yeast growth must not be overlooked. This is still more probable in the cases in which only 0·25 g. of pressed yeast was taken and tested with yeast extract itself, various precipitates from yeast extract, and with sodium nucleinate or ammonium formate [1912, pp. 216, 217; Euler and Cassel, 1913], in a total volume of 40 cc.

An experiment made on this point showed that under similar conditions of concentration growth readily occurs at 25°. The yeast was added as 5 cc. of a suspension of 5 g. yeast in 100 cc. H_2O, i.e. 0·25 g. yeast.

(1) and (2) 5 cc. yeast suspension + 20 cc. of 20 per cent. glucose + 15 cc. H_2O.

(3) and (4) 5 cc. yeast suspension + 20 cc. of 20 per cent. glucose + 15 cc. yeast extract.

In 345 mins. the evolutions were respectively 61, 59·6, 146·3, 150 cc. At the close of this time the numbers of cells present per cc. were $68{\cdot}7 \times 10^6$, $68{\cdot}5 \times 10^6$, $105{\cdot}25 \times 10^6$, $98{\cdot}8 \times 10^6$.

Asparagine acts in a precisely similar manner, 0·25 g. added to 0·5 g. yeast in 30 cc. sugar solution increasing the evolution in 2 hrs. at 25° from 73·6 to 89·4 cc.

It therefore seems that the experiments in which Euler has shown the accelerating effect of yeast extract, sodium nucleinate, etc., on the action of living yeast require revision from this point of view.

The method of testing for a co-enzyme by the action of solutions on living yeast is moreover open to the criticism that the yeast cell is, if at all, only imperfectly permeable to the co-enzyme so that negative results would be of little value.

SUMMARY.

During the alcoholic fermentation of glucose and fructose by Lebedeff's maceration extract of dried yeast, dextrorotatory polysaccharides are produced, and it is to the formation of these that the difference between the sugar removed and that equivalent to the carbon dioxide evolved is principally to be attributed.

REFERENCES.

Cremer, M. (1899), *Ber.* **32**, 2062.
Euler and Berggren (1912), *Zeitsch. Gärungsphysiol.* **1**, 203.
—— and Cassel (1913), *Zeitsch. physiol. Chem.* **86**, 122.
—— and Johannson (1912), *Zeitsch. physiol. Chem.* **76**, 347.
Harden and Young (1904), *Ber.* **37**, 1052.
—— —— (1908), *Proc. Roy. Soc.* B, **80**, 299.
Pavy and Bywaters (1907), *J. Physiol.* **36**, 149.
Slator, A. (1911), *J. Inst. Brewing*, **17**, 147.

NOTE:

HASLAM. *Separation of proteins*, Part III, Globulins. This Journal 1913, **7**, 492.

In section 4 of Summary, p. 515, *for* 0·1 mg. P $^0/_0$ *read* 0·1 P $^0/_0$.

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October MCMXIII

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Fig. 58. Chmyrëva Mogila. Golden harness adornment. ½.

Though the geographical limits have confessedly been dictated by considerations of language, yet the frontier of Russia towards the Carpathians and the Danube answers nearly to a real historico-geographical boundary, the western limit of the true steppe. The Caucasus, again, is a world in itself, having little in common with the steppe, nor has the time yet come to bring any sort of system into its archaeology. On the other hand, the unity of the Asiatic and European steppe has led the author on occasion right across to Siberia, Turkestan and China.

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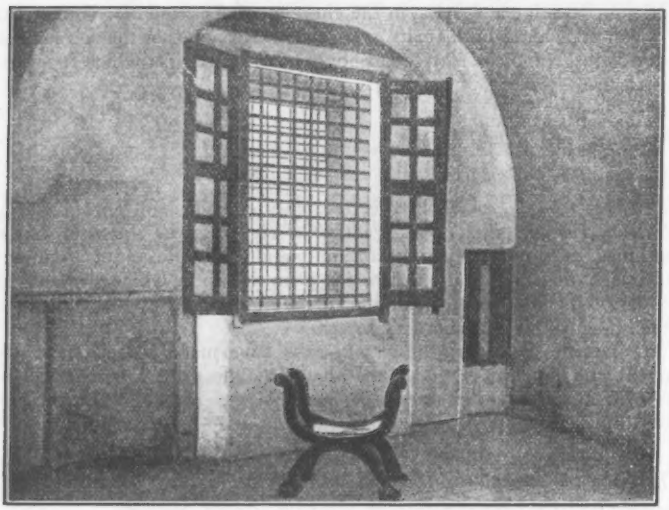

The Cell of "The Man with the Iron Mask"

gap in English theological literature. After reviewing the islands themselves, the lives of S. Vincent and S. Patrick, and the foundation of the monastery, the author treats of the monastery as a "Nursery of Bishops," and in an appendix deals with Ancient Buildings, Treasures and Monuments.

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The Cambridge History of English Literature. *Edited by Sir A. W. Ward, Litt.D., F.B.A., Master of Peterhouse, and A. R. Waller, M.A., Peterhouse. Volume X, The Age of Johnson.*

Royal 8vo. pp. xvi+562. Price in buckram, 9*s.* net, in half-morocco, 15*s.* net; to subscribers 7*s.* 6*d.* net and 12*s.* 6*d.* net respectively

As was hinted by the editors in the *Prefatory Note* to volume IX, the canvas of English literature grows more and more crowded as the eighteenth century approaches. The present volume is entitled *The Age of Johnson*, and the contents are as follows:

Richardson (L. Cazamian). *Fielding and Smollett* (Harold Child). *Sterne, and the Novel of his Times* (Professor C. E. Vaughan). *The Drama and the Stage* (Professor G. Nettleton). *Thomson and Natural Description in Poetry* (A. Hamilton Thompson). *Gray* (The late Rev. Duncan C. Tovey). *Young, Collins and Lesser Poets of the Age of Johnson* (Professor George Saintsbury). *Johnson and Boswell* (David Nichol Smith). *Oliver Goldsmith* (Henry Austin Dobson). *The Literary Influence of the Middle Ages* (Professor W. P. Ker). *Letter-Writers, I* (H. B. Wheatley); *II, The Warwickshire Coterie* (The Ven. Archdeacon W. H. Hutton). *Historians, I, Hume and Modern Historians* (The Rev. William Hunt, D.Litt.); *II, Gibbon* (Sir A. W. Ward). *Philosophers* (Professor W. R. Sorley). *Divines* (The Ven. Archdeacon W. H. Hutton). *The Literature of Dissent*, 1660–1760 (William Arthur Shaw). *Political Literature*, 1755–1775 (C. W. Previté-Orton).

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Desert and Water Gardens of the Red Sea. *Being an account of the natives and the shore formations of the coast. By Cyril Crossland, M.A. Cantab., B.Sc. Lond., F.L.S., F.Z.S., Marine Biologist to the Sudan Government.*

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The portion of the Red Sea coast described in this book (that between 18° N. and 22° N. on the western side) is, says the author, one of the least known coast-lines in the world. The country is a desert with a sparse population of nomads; no steamer passes within miles of the outermost reefs; and native vessels sail by at the rate of about one a month.

Arabian Sword Dance

The fact, moreover, that the country is only artificially made habitable at all adds interest to the author's narrative in which information of general interest, as well as a description of things and peoples peculiar to the country, is included.

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In a postscript to his preface the author finds that his book has a moral, a thing never intended! "It is that real romance and beauty are to be found in things as they are, so that the man of science, popularly supposed to be hardened by 'materialistic' pursuits, has opportunities for a truer worship than has the sentimentalist who bows before idols of his own imagination."

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"On my return from Western China in September 1910, I settled down to humdrum life with every prospect of becoming a quiet and respectable citizen of Shanghai. But...travel had bitten too deeply into my soul and...when after months of civilized life something better turned up, I accepted with alacrity. This was none other than the chance of plant-collecting on the Tibetan border of Yun-nan."

The results of this expedition are recorded in the book and a preliminary list of 200 plants collected (several of them being new species) is given in an appendix; but Mr Ward studied men and manners as well as plants. Here is part of his description of a Tibetan festival:

"The Tibetans always strike me as being so much more jolly and irresponsible than the Chinese. ...The children picked bunches of flowers just as English children love to do, romped, made swings, and swung each other and finally sat down to eat cakes....In the evening they all trooped back to the village to dance in the mule square and skip. Three or four little girls would link arms and facing another similar line of girls advance and retreat by turns, two steps and a kick, singing a not unmusical chorus—a most delightful parody of 'Here we go gathering nuts and may'."

A Tibetan girl of A-tun-tsi

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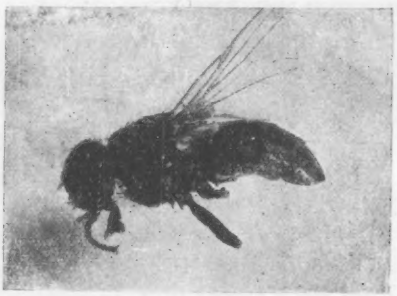

Photograph (side view) of an unfed fly (×7)

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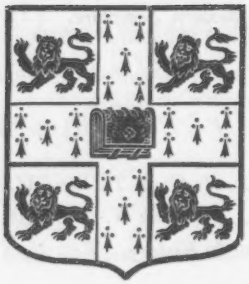
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